#3015

COMPENDIUM OF THERMAL EFFECTS LABORATORY STUDIES

Submitted to:

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77 Beale Street
San Francisco, CA 94106

April 1982

B-81-403

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PREFACE

This compendium incorporates reports prepared during 1978 to 1980 from thermal effects research on seaweeds, invertebrates, and fishes conducted at the Diablo Canyon Power Plant onsite marine laboratory. The findings of these reports are intended to complement field studies designed to detect changes in marine communities of Diablo Cove that will be exposed to the power plant thermal discharge.

Marine environmental studies at Diablo Canyon have been ongoing since 1966. In 1975, TERA prepared a research plan for Pacific Gas and Electric Company to conduct thermal effects investigations on the thermal tolerances of representative marine species to meet certain regulatory requirements associated with the power plant's discharge permit. Much of the information recommended by the EPA Regulatory Guidelines (316a Demonstration Program) could not be obtained from field observations or from the existing literature. Because of the scope of work and the special requirements for maintaining marine organisms in a laboratory, a large circulating seawater laboratory was required. Design and construction of the laboratory and associated facilities commenced in August 1976 and was completed in October 1977. The scientific staff then began operating and testing the equipment. The thermal effects experiments completed in the laboratory from 1978 to 1980 (including those previously reported) are reported in their entirety in this compendium.

1.0 INTRODUCTION

This document is a compendium of reports on the results of thermal effects laboratory experiments conducted at Pacific Gas and Electric Company's (PGandE) Diablo Canyon Power Plant site. The purpose of this research was to provide information on the thermal responses under laboratory conditions of marine fauna and flora occurring in Diablo Cove.

From May 1978 to May 1980, about 100 experiment runs were completed on 45 species of marine organisms occurring in Diablo Cove (see TABLE 1-1). These included eight fishes, 28 invertebrates, eight seaweeds and one seagrass. These experiments were conducted in a large seawater laboratory (the Thermal Effects Laboratory) built on the power plant site specifically for the research presented in this report. A full description of the 6,000-sq ft laboratory, including the automated environmental control systems, is presented in Appendix A.

This information has been used to produce a predictive assessment of the biological effects of the power plant thermal discharge (TERA 1982) and it will be useful in interpretating field data which will document changes in the Diablo Cove fauna and flora after operation of the power plant.

The advantage of laboratory experiments is that they establish cause-and-effect relationships under controlled conditions, providing a theoretical foundation for understanding potential changes in natural populations. Furthermore, certain subjects (for example, the effects of temperature on reproduction or growth) can be investigated easily in the laboratory, whereas in the field it may be impractical or impossible to obtain similar information.

Although the ultimate objective of these experiments is to aid interpretation and assessment of changes in natural populations, it is not always clear how laboratory results can be applied to a natural population even when the results are known to be precise and repeatable in the laboratory. There are two fundamental reasons for this: by definition, all laboratory experiments are

TABLE I-1

THERMAL EFFECTS EXPERIMENTS COMPLETED FROM 13 MAY 1978 TO 4 MAY 1980 AT THE DIABLO CANYON THERMAL EFFECTS LABORATORY

Species/Experiment	Report Published**
HEAT TOLERANCE	
Botryoglossum 96h LT50	N
Calliarthron 96h LT50	N
*Gastroclonium 96h LT50	N
*Iridaea flaccida 60D "LT50" (gradient)	F
*Iridaea flaccida 60D "LT50" (raceway)	F
*Nereocystis 60D "LT50" (raceway)	N
*Pterygophora 30D "LT50" (raceway)	N
Phyllospadix scouleri 216h LT50	
Embiotica jacksoni 96h LT50	F
Micrometrus aurora CTM	
Scorpaenichthys marmoratus CTM	
*Sebastes chrysomelas/carnatus 96h LT50	F
*Sebastes mystinus 96h LT50	N
Sebastes serranoides 96h LT50	N
*Xiphister mucosus CTM (high temp. accl.)	
*Xiphister mucosus 96h LT50	A
Acanthina spirata 96h ET50	
Acmaea mitra 96h ET50	N
Astraea gibberosa 96h ET50	N
*Cancer antennarius 96h ET50	A
Colisella digitalis 96h ET50	
C. limatula 96h ET50	
C. pelta 96h ET50	
C. scabra 96h ET50	
*Haliotis cracherodii 96h LT50	F
*Haliotis cracherodii (high temp. accl.)	N
*H. rufescens (high temp. accl.)	N
Hemigrapsus nudus 96h ET50	A
Leptasterias hexactis 96h ET50	
Ocenebra circumtexta 96h ET50	
Pachygrapsus crassipes 96h ET50	A
Pagurus granosimanus 96h ET50	A
Pagurus samuelis	A
Patiria miniata 96h ET50	
Petrolisthes cinctipes 96h ET50	A
Pisaster brevispinus	
Pisaster giganteus 96h ET50	
*Pisaster ochraceus 96h ET50	
*Pugettia producta 96h ET50	A
Pugettia richii 96h ET50	A
Strongylocentrotus purpuratus 96h ET50	
*Tegula brunnea 96h ET50	N
*Tegula brunnea (high temp. accl.)	
T. funebralis	N
Tonicella lineata 96h ET50	N

**Diablo Canyon 316(a) Nine-Month Progress Report (F = February 1979, N = November 1979, A = August 1980)

* Designated an important species.

TABLE I-I

THERMAL EFFECTS EXPERIMENTS
(CONT.)

Species/Experiment	Report Published**
TEMPERATURE PREFERENCE AND OPTIMUM FOR GROWTH	
Botryoglossum (raceways)	A
*Iridaea flaccida (gradient)	F
*Iridaea flaccida (raceways)	F
*Laminaria dentigera (gametophytes-gradient)	A
*Nereocystis (gametophytes - gradient)	N
*Nereocystis (sporophytes - raceways)	N
*Pterygophora (gametophytes - gradient)	A
*Pterygophora (sporophytes - raceways)	A
Embiotoca jacksoni (juveniles - preference)	N
Embiotoca jacksoni (subadults - preference)	
Embiotoca jacksoni (growth)	
Scorpaenichthys marmoratus (preference)	
*Sebastes chrysomelas/carnatus (juveniles - preference)	
*Sebastes chrysomelas/carnatus (subadults - preference)	
*Sebastes chrysomelas/carnatus (growth)	A
*Sebastes mystinus (juveniles - preference)	
*Sebastes mystinus (subadults - preference)	
*Sebastes mystinus (growth)	A
*Xiphister mucosus (preference)	
*Xiphister mucosus (growth)	A
Astraea gibberosa (preference)	
*Haliotis cracherodii (preference)	
*Haliotis cracherodii (growth)	N
*Haliotis rufescens (preference)	
*Haliotis rufescens (growth)	
Leptasterias hexactis (preference)	
Pagurus granosimanus (preference)	
Pagurus samuelis (preference)	
Patiria miniata (preference)	
Pisaster giganteus (preference)	
*Pisaster ochraceus (preference)	
*Pugettia producta (preference)	
*Pyncopodia helianthoides (preference)	
*Tegula brunnea (preference)	
*Tegula brunnea (growth)	
Tegula funebralis (preference)	
Tegula funebralis (growth)	
MINIMUM, OPTIMUM AND MAXIMUM TEMPERATURE FOR EARLY DEVELOPMENT	
*Iridaea cordata (gametophytes)	A
*Iridaea flaccida (gametophytes)	A
*Iridaea flaccida (sporophytes)	A
*Laminaria dentigera (gametophytes)	A
*Laminaria dentigera (sporophytes)	A
*Nereocystis (gametophytes)	F
*Nereocystis (sporophytes)	N

**Diablo Canyon 316(a) Nine-Month Progress Report (F = February 1979, N = November 1979, A = August 1980)

* Designated an important species.

TABLE I-I

THERMAL EFFECTS EXPERIMENTS
(CONT.)

Species/Experiment	Report Published**
MINIMUM, OPTIMUM AND MAXIMUM TEMPERATURE FOR EARLY DEVELOPMENT (CONT.)	
*Pterygophora (gametophytes)	N
*Pterygophora (sporophytes)	A
Scorpaenichthys marmoratus egg hatching	
Scorpaenichthys marmoratus larval development	
*Haliotis cracherodii egg-embryo development	A
*Haliotis cracherodii larval development	A
*Haliotis rufescens egg-embryo development	A
*Haliotis rufescens larval development	A
*Haliotis rufescens settlement	A
PREY CAPTURE/PREDATOR AVOIDANCE	
*Cancer/Haliotis/Tegula 48h test	
*Pisaster/Haliotis/Tegula 48h test	

**Diablo Canyon 316(a) Nine-Month Progress Report (F = February 1979, N = November 1979, A = August 1980)

* Designated an important species.

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conducted under conditions different from those in the natural environment, and secondly, in nature an organism responds to a multiplicity of stimuli to which it is simultaneously exposed. Therefore, it is important to remember in applying laboratory results to the natural world that many of the conditions found in nature cannot be simulated accurately in the laboratory. We found in our research that this was particularly true with respect to such natural marine phenomena as tides, winds and sunlight. In the laboratory, efforts were made to reproduce sunlight conditions by fluorescent lighting and tides were simulated by the use of special pumps to change water levels in holding tanks. By simulating these conditions we were able to gain some insight into the importance of light levels and tides, but were unable to truly simulate the magnitude and the subtlety of changes found in nature.

We believe that the laboratory results should be used cautiously in extrapolating expected or anticipated effects of the thermal discharge. The principal caution in applying the laboratory data is that the majority of the experiments were, out of necessity, conducted under a constant temperature exposure regime for long periods of time. From our temperature recordings in Diablo Cove it is quite obvious that temperatures in the natural state fluctuate greatly. In addition, the temperature of the thermal discharge will fluctuate daily and seasonally with the rise and fall of the ambient water temperatures. An intertidal organism will be exposed to elevated discharge temperatures in this water for brief periods of time as the tide rises and falls. The effect of exposure to alternating periods of warm water and air temperature could not be completely assessed in the laboratory. In black abalone experiments, which were conducted using tidal conditions, it was quite obvious that the effect of exposure to air, partially simulating tide conditions, increased the abalone's ability to tolerate higher water temperatures.

The remaining portions of this section consist of a description of the power plant setting, a history of the regulatory background related to these studies and an outline of the report organization.

1.1 POWER PLANT DESCRIPTION AND SETTING

The Diablo Canyon Power Plant (DCPP) is located on a 750-acre site on the California coast midway between Point Buchon and Point San Luis (see FIGURE 1-1). The site is approximately 12 miles (19.3 km) west of the City of San Luis Obispo and approximately midway between San Francisco and Los Angeles.

The power plant, which is not operating but is nearly completed, is a two-unit, nuclear-fueled steam electrical power plant. Each unit will use a four-loop, pressurized-water nuclear reactor designed for baseload operation with a net capacity of 1,086 MWe for Unit 1 and 1,119 MWe for Unit 2. Commercial operation of Unit 1 is expected to commence in 1982.

The major facilities at the site are two reactor containment structures, two fuel handling buildings, a common auxiliary building, and a turbine building housing the two turbine-generator units. Cooling water for the two units will be drawn from a common structure located in an intake cove, pumped approximately 130 ft uphill to the power plant and returned by a cascade shoreline discharge structure to the Pacific Ocean.

Each unit has two condensers serviced by two circulating water pumps which produce a combined flow from Unit 1 condensers of between 778,000 gpm and 854,000 gpm and from Unit 2 condensers of between 811,000 gpm and 895,000 gpm. The combined two-unit measured flow is 1,589,000 gpm minimum and 1,749,000 gpm maximum. After passing through the condensers, the cooling water flows through conduits to the discharge structure located on the shoreline cliffs of Diablo Cove. The removal of heat from the units to condense steam (15.2×10^9 Btu/hr for both units at full design power) will result in a cooling water discharge temperature about 20°F warmer than the incoming seawater temperature. The temperature of the thermal discharge will vary as the incoming ocean water temperature warms and cools with the seasons.

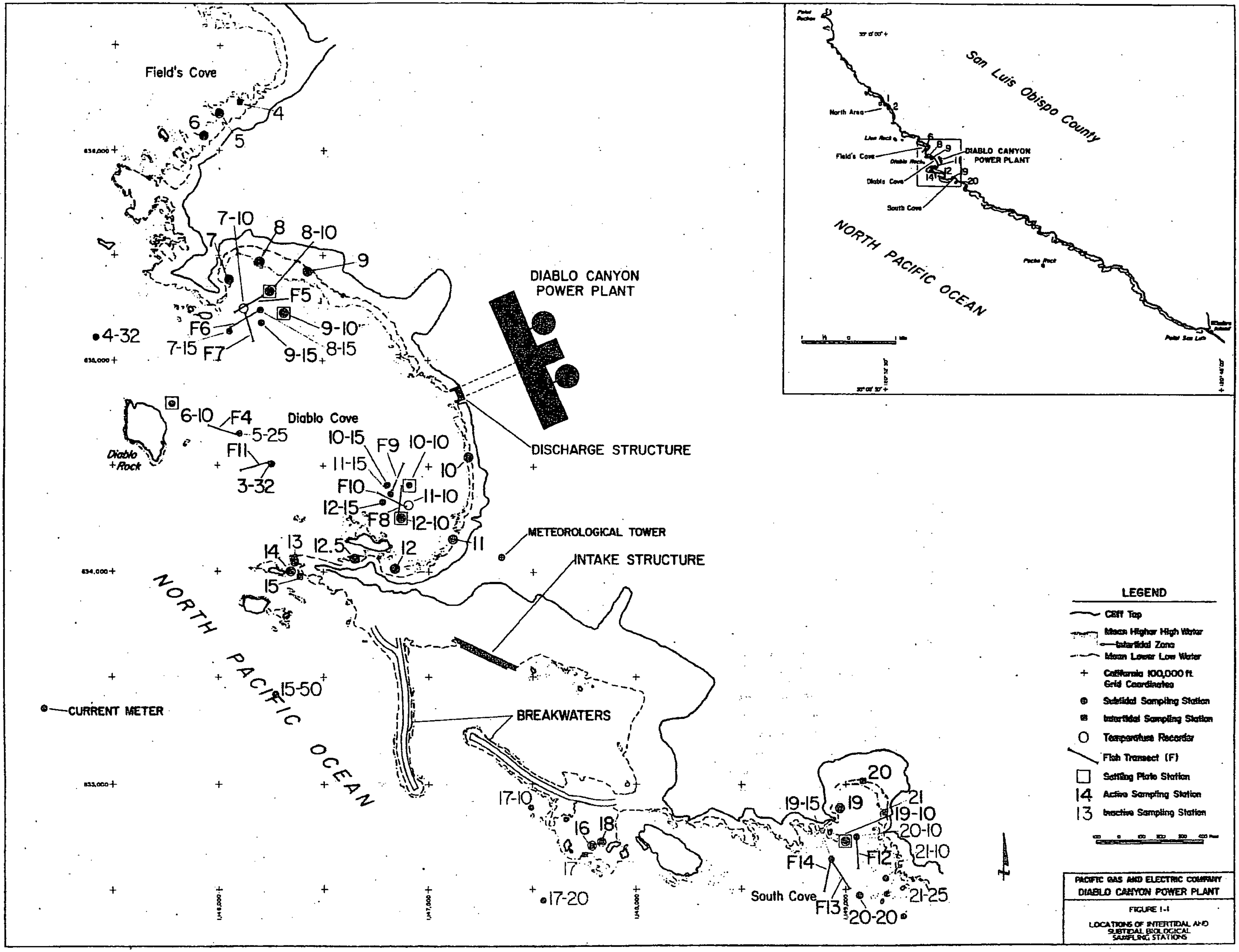

FIGURE 1-1

LOCATIONS OF INTERTIDAL AND SUBTIDAL BIOLOGICAL SAMPLING STATIONS

The average discharge water temperature will range between 70°F and 77°F; the cooler temperatures will occur between February and July, and the warmer temperatures from August to January.

1.2 BACKGROUND

Marine environmental studies at Diablo Canyon have been ongoing since 1966. One such study, which has been regularly conducted by Dr. Wheeler J. North since 1966, has provided an extensive inventory of intertidal and subtidal species. The duration and complexity of these studies reflect the importance which the responsible state agencies have placed on such studies. For example, the 1966 agreement with the California Resources Agency stressed the need for a comparison between baseline environmental studies and studies conducted after DCPP commences operation. In addition, the 1969, 1974 and 1976 permits issued by the California Regional Water Quality Control Board, Central Coast Region (Regional Board) allowed the commercial operation of DCPP but required a thermal effects report to be submitted after operation commences.

In 1975, Pacific Gas and Electric Company (PGandE) submitted a plan for a thermal effects study program pursuant to Section 316(a) of the Clean Water Act to the Regional Board and commenced implementation of the study plan. The study plan outlined a wide range of research strategies to assess both the general ecological effect of the power plant discharge and the effect of the discharge on a set of the 21 important species identified by the Regional Board staff and the California Department of Fish and Game. Procedures for these studies were documented and accompanied by a tabular presentation of field data in progress reports to the Regional Board dated October 1977, May 1978, February 1979, November 1979 and August 1980. A review of the literature on the 21 important species and initial results of the Thermal Effects Laboratory experiments were presented in progress reports dated February and November 1979 and August 1980.

Thousands of hours have been spent by scientists on counting, cataloging, measuring and analyzing the marine biota in Diablo Cove and the surrounding area. Three principal teams, PGandE, CDF&G and PGandE's consultants, have continuously conducted research programs of the site's marine ecology since 1976 and many of the studies were begun as early as 1966. It can be confidently stated that no other discharge site on the west coast has as extensive a baseline data base as Diablo Canyon. Additionally, no other coastal discharger has constructed a marine laboratory of the size and sophistication of the DCPP thermal effects marine laboratory, specifically to study the potential effects of a proposed discharge.

At present, the marine monitoring and laboratory research initiated by implementation of the 316(a) Demonstration Study Plan is being continued as the NPDES Permit Thermal Effects Monitoring Program (TEMP). In addition to the thermal effects monitoring program, PGandE has conducted and is continuing a number of marine research programs which include intertidal and offshore fisheries studies, intertidal habitat studies, chlorine and heat studies, temperature, wave and current monitoring, thermal plume model studies and Dr. Wheeler North's historical (1966-present) transect studies. The California Department of Fish and Game has a full-time onsite marine research staff which also has been conducting independent monitoring studies since 1969.

1.3 ENVIRONMENTAL SETTING

The Diablo Canyon Power Plant (DCPP) is located on a coastal terrace near the Pacific Ocean. The area, shoreline features, and power plant site are shown in FIGURE 1-1. The coastal terrace is approximately 15 miles long, extending from Morro Bay to Avila Beach, and the DCPP is approximately in the middle of this section of coastline. To the north and south of this rocky coast, the coastline consists of sandy beaches.

The shoreline in the immediate vicinity of the plant is a series of semiprotected small coves. Heavy wave action, strong and persistent currents, and a wide variety of shoreline and seabed features produce a dynamic and complex nearshore habitat. The convoluted rocky shoreline has a high vertical relief of boulders and bedrock channels and offshore rock pinnacles. Few tidepools are found in the area due to the absence of flat bedrock shelves along the shoreline. The exposed nature of the coast allows open ocean swells of the north Pacific Ocean to produce a shoreline environment of very high wave energy. Inlets and indentations in the coastline will occasionally have cobble or coarse gravel beaches and deeper coves generally have a small sandy bar or beach.

The major portion of Diablo Cove is less than 25 feet (8 m) deep and the bottom is composed primarily of bedrock traversed by surge channels, boulders, and rock pinnnacles. Most of the intertidal and subtidal rock substrate is covered by dense growths of seaweeds and invertebrates that are commonly found along hundreds of miles of California coastline. The numerous species of large seaweeds found in the area provide habitat for other smaller seaweeds and invertebrates and food for abalone and sea urchins, which in turn are prey for rockfish and sea otters.

1.3.1 OCEANOGRAPHIC SETTING

The Diablo Canyon Power Plant (DCPP) is located on a rugged stretch of California coastline, which consists of rocky headlands interspersed with bedrock and boulder beaches. Beaches of cobble, gravel, and, in some cases, coarse sand are found in covelet areas. The coastline is exposed to the force of the open ocean swells coming from the northwest Pacific Ocean. The shoreline areas receive a great deal of wave energy that has a marked effect on both the physical and biological characteristics of the marine habitat.

The DCPP discharge enters Diablo Cove, which is a shallow indentation in the coastline, then flows into the open ocean approximately 1800 feet from the discharge point. The interior portions of the cove are less than 20 feet deep, and the underwater seabed is composed of bedrock, overlain by boulders, cobble and sand. About halfway across the cove, the bottom depth drops rapidly to a depth of approximately 60 feet at the cove entrance. Beyond the cove in the nearshore region, the seabed continues to slope downward for approximately 57 miles across the narrow continental shelf to a depth of 3000 feet. The seabed then drops rapidly in another 8 miles across the continental slope to an ocean depth of 12,600 feet.

Inside the cove the prominent bottom features include rock pinnacles, channelled bedrock, and large boulders. In the southern portion of Diablo Cove are sandy bottom areas where sand transported by local currents collect in channelled areas. During certain times of the year these sands form a small beach in south Diablo Cove.

Coastal winds are responsible for the nearshore ocean currents along the California coast. These winds, which are predominately from the northwest when a high pressure system is off the coast move the surface of the water in the general direction of the wind. The rotation of the earth causes any motion in the northern hemisphere to appear to move in a clockwise direction, i.e., to bend to the west when the motion is in the direction of the equator. This effect, known as the Coriolis force, causes the water which is moved by the wind in contact with the surface to bend in a clockwise direction. Therefore, the surface water moves to the right of the wind direction along the California coastline. With the winds blowing from north to south, the water at the surface is transported offshore. As this surface water moves offshore, it is replaced by cold bottom water coming from ocean depths of 1,000 to 3,000 feet. This cold bottom water, known as upwelling water, moves onshore and produces unusually low water temperatures along California's coast. Similar coastal wind patterns produce very cold nearshore water of a comparable upwelling volume and persist at only three areas of the world, namely, the west coast of Europe, the west coast of Africa, and the west coast of South America. June and July are the coldest seawater months of the year when upwelling is strongest along the California coast.

Water temperatures along the Diablo Cove shoreline and in the region of the DCPP discharge have been recorded for many years. FIGURE 1-2 illustrates the seasonal and annual cycles of ambient water temperatures which have been recorded from the period of 1976 to 1981. As is obvious from the temperatures illustrated in this figure, there are warm and cold seasons. These seasonal temperature declines illustrate the beginning of coastal upwelling. As the

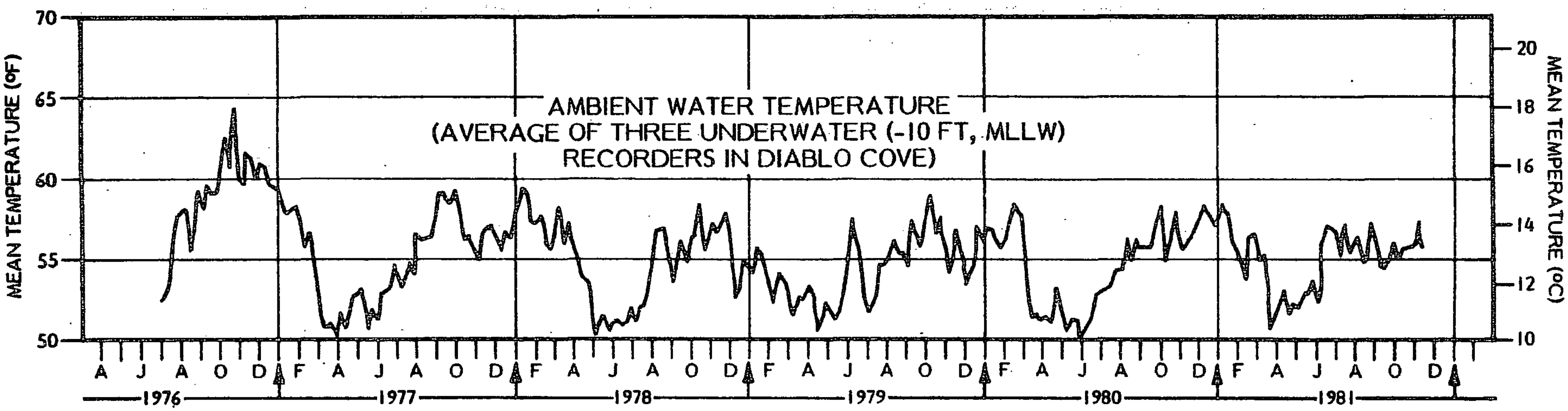

FIGURE 1-2

PLOT OF DIABLO COVE AMBIENT WATER TEMPERATURE incoming water seasonally increases in temperature, the discharge temperature will rise and fall, tracking the ambient water temperatures with a fairly constant addition of 20°F.

The currents in the vicinity of Diablo Canyon have been characterized as the California current which is a wide, slow southward surface current, and the Davidson current, a northward countercurrent. The California current is about 1,000 km wide, 500 m deep and flows about 25 cm/sec. The Davidson current flows northward at speeds between 26 and 46 cm/sec. The currents are influenced by wind patterns in the northeast Pacific Ocean. Because the prevailing winds tend to occur in seasonal patterns, the currents vary accordingly. Three ocean seasons, related to the major coastal currents are characterized by individual hydrographic conditions, have been described for the California coast. These are the oceanic, Davidson, and upwelling seasons, which differ significantly from the terrestrial seasons.

1.3.2 BIOLOGICAL SETTING

The assemblages of plants and animals found in Diablo Cove are commonly referred to as communities. Ecologists commonly define a "community" by the presence and absence of key species, which by their abundance and predictable association determine the characteristics of the community. However, the interactions among the species (competition, predation, etc.) determine the community's structure, that is, a web of connections is established among the species. In physically demanding and changing environments, (such as open coast), the ecological community is strongly influenced by the environment's physical characteristics and community interactions are minimal.

The communities of marine plants and animals found along California's open ocean coastline are comprised of species with special adaptations to colonize, grow and reproduce in the rigorous shoreline habitat. Large waves continually pound the shore and dislodge or erode all but the most securely attached or protected species. These communities are derived from and maintained by an essentially infinite supply of larval and juvenile shoreline species carried by the large longshore currents that continually bathe the coastal habitats. Various habitats intercept this flow of space-seeking larvae and juvenile species; and, if space is available in the habitat, the physical characteristics (wave action, amount of tidal exposure, substrate stability, light, and temperature) and biological characteristics (predation) of the habitat will select from the coastal current supply of larvae, those species which can survive in the habitat. Most marine plant and animal species reproduce by drifting larval forms, plankton, which are carried by ocean currents over hundreds of miles, and thereby greatly increase the chances that their offspring will find suitable open spaces to settle. Unlike a tropical reef marine habitat where physical characteristics are benign and the community structure is determined by complex biological interactions (predation/competition), the structure of the marine community along the California coast (and Diablo Cove) is weakly developed and is secondary to the characteristics related to physical properties of the habitat.

However, biological interactions, such as predation, habitat formation and competition for light, determine the Diablo Cove community structure. For example, sea urchins and abalone grazing on seaweeds have had a measured and significant effect on the abundance and distribution of forage seaweeds. In Diablo Cove and elsewhere there is evidence that competition for light and shadowing on the bottom by surface kelp canopies contribute to the abundance and distribution of underwater seaweeds.

The waveswept, rocky shoreline and underwater areas of Diablo Cove provide diverse habitats for a wide variety of marine plants, invertebrates and fishes. Many of these organisms are characteristic of the cool, temperate coastal zone that streches from Pt. Conception northward to British Columbia. To provide an overview of the diverse plant and animal communities inhabiting Diablo Cove, a line perpendicular to the shore will be described starting in the high intertidal area (a shoreline zone occasionally exposed by the changing tides), continuing through the wave surge of the lowest intertidal zone and ending in the shallow underwater regions of Diablo Cove. Such a vertical cross-section of the cove's plant communities is illustrated in FIGURE 1-3. This figure is discussed below.

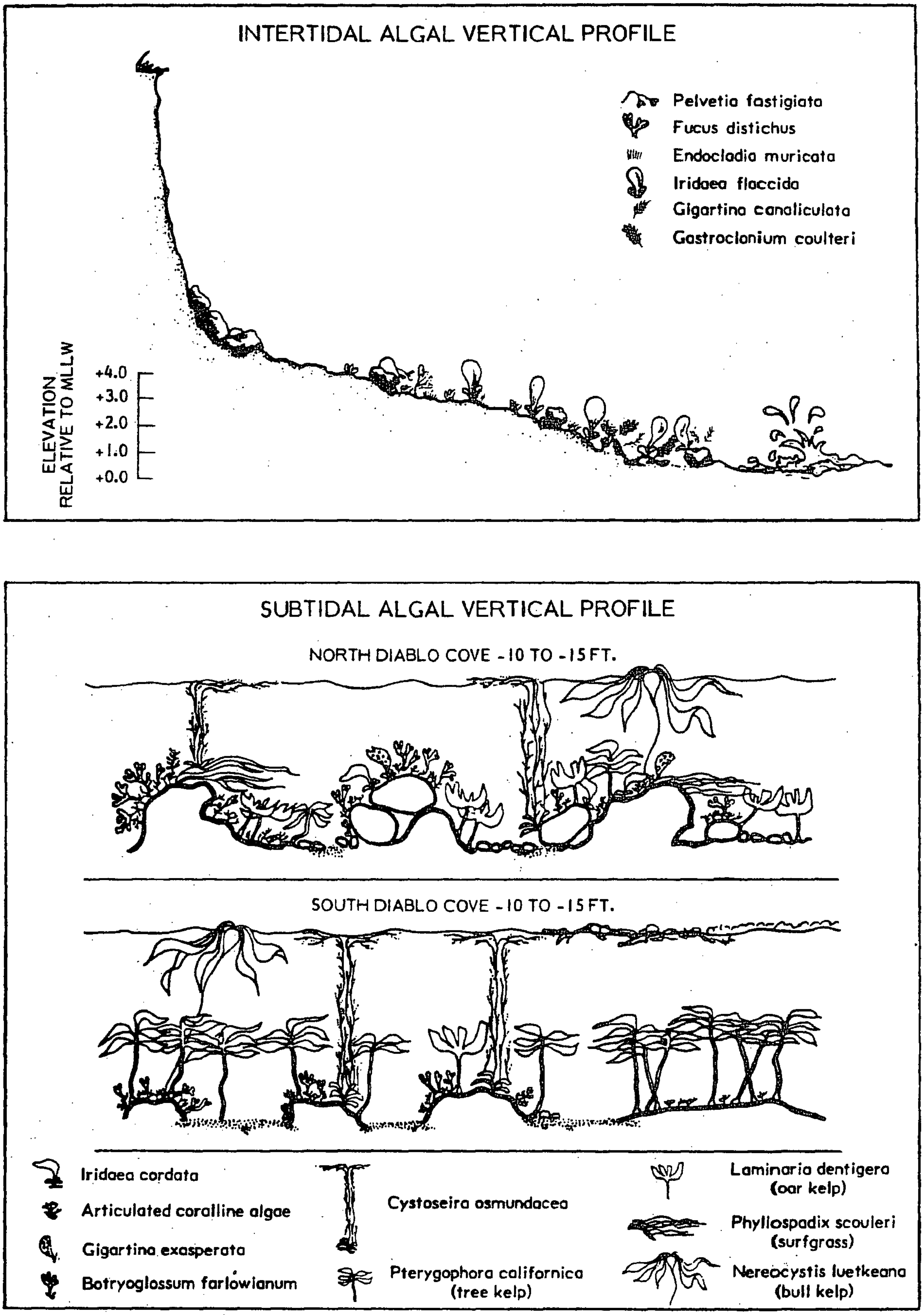

FIGURE 1-3

PROFILES OF DIABLO COVE
INTERTIDAL AND SUBTIDAL PLANT COMMUNITIES

On the rocky headlands, which define the outermost boundaries of Diablo Cove, an assemblage of organisms particularly well adapted to the rigors of extreme wave exposure is found. The California mussel, goose-necked barnacle and sea palm (a brown kelp) are some of the firmly attached inhabitants characteristic of this zone. The ochre sea star can usually be observed feeding on the mussels as they pry apart the mussel's shell. Such biological interactions are essential in determining the presence and abundance of the various organisms in the cove. As the sea star removes the mussel from the rock bench, new space becomes available for colonization by juvenile mussels, barnacles or fast growing seaweeds. Every square inch of stable rock surface is likely to be occupied by a kelp holdfast, encrusting sponge, tube worm, barnacle or another organism. This competition for living space, another biological interaction, also determines to a large extent the types of species present in Diablo Cove.

A closer look at the intertidal zone reveals a luxurient growth of red, brown and green seaweeds which overlay the boulders and bedrock. Among the most common varieties of seaweed are the iridescent seaweed, grapestone seaweed, rockweed, sea lettuce and crustose forms adhering closely to the rocks. These seaweeds provide both food and shelter for grazing snails and limpets in the intertidal zone. However, the thousands of turban snails, hermit crabs and black abalone in this surf zone prefer to feed on seaweed which has been ripped from the substrate by wave action and cast ashore as drift (detritus). Tons of drift seaweed which may originate far to the north are present along the shore during the fall and winter seasons after violent storms. The seaweeds have become well adapted to this annual cycle by growing rapidly in the spring and summer as calmer sea conditions and increased sunlight and nutrient-rich upwelling water provide favorable growing conditions.

Extending seaward from the intertidal zone is the zone of green surfgrass which encircles Diablo Cove to a depth of several feet. The long, narrow leaves and densely intertwined root systems of the surfgrass are habitat for minute snails, polychaete worms, brittle stars, encrusting tunicates and small crustaceans. In addition, juvenile crabs and fish take refuge in these stands of surfgrass. This variety of potential prey items attracts predatory fish such as the striped surfperch, pile surfperch, greenling and sculpin.

At greater depths in the Diablo Cove subtidal region, the sea bed is characterized by dense stands of fleshy red algae and tough corallines, forming an understory layer, and larger brown kelps forming a light-shading canopy cover. Where channels of sand traverse the bedrock and boulders, these plants are unable to secure themselves to the substrate. As in the intertidal zone, competition for attachment space is intense, resulting in rock surfaces which are overgrown by a multitude of sponges, tunicates, sea anemones, sea stars and tube worms. Red abalone, sea urchins and crabs may also be found in rock crevices or between boulders.

Another conspicuous species of the underwater biological community in Diablo Cove is the bull kelp, a fast growing kelp that forms an extensive surface canopy in the summer and fall. Blue rockfish, surfperch and juvenile fish of many species use this canopy for food and protection. Occasionally, a sea otter can be spotted within the surface canopy feeding on a recently caught abalone or rock crab.

Within Diablo Cove there are subtle differences in the general biological patterns. These noticeable and persistent features of the cove become obvious after many years of observing the same communities. Many of the differences are related to wave exposure and to the types of substrate in different areas of the cove. These differences are illustrated schematically in FIGURE I-3 which compares the underwater plant communities in north and south Diablo Cove. Accumulation of sand in south Diablo Cove due to the cove's current patterns fills in the bedrock channels and removes the hard, stable substrate which the plants need for attachment. These sands also fill the water when the waves are high and can severely scour and erode plants and animals. The combined effects of sand and wave action result in the reduced abundance and diversity of plants in south Diablo Cove.

Other small patterns in the communities of Diablo Cove are not understood. Black abalone are much more abundant in the intertidal areas of the north and south points of Diablo Cove than in similarly appearing habitat inside the cove. Annual changes in the abundance and distribution of bull kelp vary dramatically from a near absence in the cove to such an abundance that it is difficult to navigate in the cove. Reasons for these changes in the cove's kelp beds are not apparent, though similar changes occur in kelp beds in southern California.

In summary, the complex varieties of marine life in and around Diablo Cove are well adapted to the physical stresses of strong wave action and fluctuating tides, and the biological stresses of competition for space and predation. The question of how this community will be altered or affected by the addition of warm water discharge is one which requires a detailed examination of the major component species of the community and an assessment of their physiological response to water temperature changes.

1.4 REPORT ORGANIZATION

The experiment results are presented in three major categories based on the thermal index studied. In Volume 1, SECTION 2 includes the results of heat tolerance experiments on 41 species, i.e., the responses to relatively short-term (typically 96-hour) exposure to relatively high temperatures. In Volume 2, studies of effects of temperature on growth and early development of 16 species is presented in SECTION 3. Behavioral response to temperature studies, including preference, avoidance, escape, and species interaction are presented in SECTION 4. SECTION 5 includes the literature cited in the foregoing sections and an acknowledgement to those individuals who assisted in the research. The appendices are contained in Volume 3. APPENDIX A is a description of the Thermal Effects Laboratory facilities and data management system, APPENDIX B includes detailed procedures for all experiments conducted, and APPENDIX C includes all protocols for each run of an experiment.

2.0 HEAT TOLERANCE

The purpose of heat tolerance experiments is to determine the short-term effects of increased temperature on organisms as measured by either physiological or "ecological" death. "Ecological" death is defined by a behavioral criterion which is considered to be essential for survival of the organism under natural conditions and is used when it is impossible to determine by observation that an individual has died. These acute temperature effects are assessed on animals through 96-hour median lethal temperature tests and, for fish, by critical thermal maximum (CTM) bioassays. Nationally Standardized procedures for these tests have been published (Standard Methods 1975) and were followed in the experiments reported in this chapter, except insofar as they could be improved upon for tests with marine organisms. The results of these experiments are used to determine a species' upper lethal temperature and the time course of mortality in test populations subjected to relatively high temperatures for short periods of time. Upper lethal values can be used to define temperature limits which species can survive for only short exposures, and the time course of mortality may be used to predict the survivorship of organisms subjected to abnormally high temperature conditions for short periods. Results of short-term heat tolerance tests can be used to design additional experiments which would better define sublethal or chronic responses to elevated temperatures. In many cases the results of heat tolerance experiments may be applied directly to predict the effects of the discharge on a species, depending on a particular population's distribution in both time and space.

This section reports the results of heat tolerance experiments on six species of fishes, twenty-seven species of invertebrates, and six species of algae.

2.1 BLACK-AND-YELLOW/GOPHER ROCKFISH (SEBASTES CHRYSOMELAS/CARNATUS)

Juvenile specimens of some species of rockfish (genus Sebastes) are impossible to identify to species. Juveniles similar to those used in these experiments are frequently observed in the nearshore areas of Diablo Canyon and can be assigned with reasonable certainty to either S. chrysomelas (black-and-yellow rockfish) or S. carnatus (gopher rockfish). The geographical distribution of these species extends from Eureka, California to Baja California, Mexico (Miller and Lea 1972). They reach a maximum length of 39 cm (15 in.).

The gopher rockfish is an important species in the ecology of Diablo Cove. The literature on it and on the black-and-yellow rockfish was reviewed in a previous report (PGandE 1979b).

Two types of heat tolerance experiments were conducted on black-and-yellow/gopher rockfish juveniles. 96-hour median lethal (LT50) tests and critical thermal maximum (CTM) tests were conducted on 12 and 16 C-acclimated animals.

2.1.1 96-HOUR MEDIAN LETHAL TEMPERATURE (96 H LT50)

The purposes of LT50 experiments on juvenile black-and-yellow/gopher rockfish were:

- To observe the time course of mortality for groups of fish exposed to heated water over a 96-hour period;
- To determine from these observations the temperatures at which 50% of the fish died (LT50 values) for standardized 96-hour exposures and for shorter exposures;
- To measure the effect of acclimation to warm (16C) and cold (12.2C) temperatures on the LT50 values for the fish.

The two acclimation temperatures were selected to represent average warm season and cold season sea-water temperatures at the Diablo Canyon coast, so that seasonal changes in the thermal tolerance of the fish could be assessed.

2.1.1.1 METHODS

A summary description of the laboratory methods employed in the LT50 tests of juvenile black-and-yellow/gopher rockfish is presented below. For a detailed description of the laboratory methods see APPENDIX B (Procedure 305) and protocols see APPENDIX C (experiments 5e to h). Juvenile black-and-yellow/gopher rockfish were collected using a 3 m (loft) otter trawl during July and August 1978, from an area in the outer region of Morro Bay, just north of Diablo Cove. The fish were immediately transferred to the thermal effects laboratory at Diablo Canyon and held in ambient temperature seawater prior to acclimation. Holding tanks were raised or lowered 1C per day until the proper acclimation temperature (12 or 16C) was reached. The fish were then held at this temperature until acclimated.

The length of acclimation period was determined by the use of critical thermal maximum tests (CTM) which monitored changes in the fish thermal tolerance levels (see SECTION 2.1.2). In the CTM tests four fish were removed from the acclimation tank and placed in a small aquarium with an immersion heater. The temperature in the tank was raised at a constant rate of approximately 7C per hour, and the temperature at which the fish expired was recorded (the mean temperature at mortality of four fish equals the CTM). Theoretically, a fish acclimating to a warmer temperature should exhibit an increasing tolerance to elevated temperatures and, thus, a higher CTM. The rate of increase in CTM temperatures levels off when the fish are fully acclimated to the new temperature (a matter of hours or days depending on the species and the temperature differential involved). The minimum acclimation period during the 96-hour LT50 experiment was 6 days.

Following acclimation, 10 individuals were transferred to each of six experimental tanks and held for 12 to 24 hours at their acclimation temperature to allow them to adjust to their new surroundings. At the start of the experiment, one tank remained at the acclimation temperature while the other five tanks were raised to their various elevated test temperatures, within one hour if possible (some of the warmer tanks required a slightly longer period for temperature adjustment, but all temperatures were reached within 2 hours). Observations were made every hour for the first six hours, and following this period the fish were observed at 18, 30, 42, 54, 66, 78, 90 and 96 hours. Dead fish were removed and measured, and behavioral notes were made as part of the observations. Following the 96-hour period the tanks were returned to the acclimation temperature, and a final set of observations was made 24 hours later. The experiments were repeated twice for fish from each of the two acclimation temperatures. The first experiment involved a wider range of test temperatures than the second and the results were used to select a narrower range of temperatures used in the second experiment. The time between experiments ranged from 1 to 8 days.

Test temperatures were controlled and recorded on an hourly basis by an Autodata Nine temperature controlling and data acquisition system described in APPENDIX A. Mean experimental temperatures were calculated from hourly readings for the 96-hour test period or for that period leading up to 100 percent mortality of a group of fish. When the time to 100 percent mortality was substantially shorter than 96 hours, the variability of the test temperature was strongly influenced by the initial temperature adjustment period and resulted in relatively higher standard deviations about the mean. Probit analysis of the mortality data was carried out in accordance with procedures specified in Standard Methods (1976). Two consecutive experimental runs were conducted on fish acclimated to each of the two temperatures (12.2C and 16.0C).

2.1.1.2 RESULTS

Juvenile black-and-yellow/gopher rockfish acclimated to 12.2 and 16.0C were exposed to various test temperatures ranging from 23.9C to 27.5C, and the time courses of the mortality of two experimental runs on each acclimation group are shown in TABLES 2.1-1 and 2.1-2, respectively. For each observation period, cumulative mortalities are plotted on a probability scale versus their respective mean test temperatures. (Example plots are shown in FIGURE 2.1-1 of the 5 and 96-hour observations of the 16C acclimated fish and in FIGURE 2.1-2 for the 7, 8 and 9-hour observations of the 12.2C acclimated fish.) A line fitted by eye to each of the plots was used to extrapolate the temperature at which exactly 50 percent mortality of each group of fish would occur. In each acclimation group, the extrapolated temperatures which caused 50 percent mortality were then plotted for each observation period (FIGURE 2.1-3). The temperature at which

TABLE 2.1-1

PERCENTAGE THERMAL MORTALITY VERSUS TIME (96 HRS)
IN 12C-ACCLIMATED BLACK-AND-YELLOW/GOPHER ROCKFISH JUVENILES

Species: Sebastes chrysomelas/carnatus   Acclimation Temperature: 12.2C

$\bar{X}$ Standard Length: 48.8 mm.
$\bar{X}$ Weight: 3.2 g.

Experiment 5f   11-16-78 through 11-20-78

$\bar{X}$ Experiment Temperatures, C:	12.2	22.0	24.0	24.9	25.8	27.4
Standard Deviations:	0.05	0.05	0.05	0.36	0.50	0.78

Experiment 5h   11-29-78 through 12-03-78

$\bar{X}$ Experiment Temperatures, C:	12.2	22.1	22.5	22.9	23.5	24.0
Standard Deviations:	0.05	0.04	0.02	0.02	0.09	0.15

Zero Mortality, C: 12.2, 22.0, 22.1, 22.5

PERCENTAGE MORTALITY

Experiment Code		5h	5h	5h	5f	5f	5f	5f
Temperature, C		22.9	23.5	24.0	24.0	24.9	25.8	27.4
Time (hours)	1						20	100
	2			10	10		40	
	3		10			20	90	
	4			30	20		100	
	5					70		
	6			50	40	100		
	7			60				
	8		20	70				
	9		40	80				
	10	10		100				
	11							
	12		50					
	18				100			
	30							
	42							
	54	20						
	66							
	78							
	90							
	96							
Total Percentage Mortality		20	50	100	100	100	100	100

TABLE 2.1-2

PERCENTAGE THERMAL MORTALITY VERSUS TIME (96 HRS)
IN 16C-ACCLIMATED BLACK-AND-YELLOW/GOPHER ROCKFISH JUVENILES

Species: <u>*Sebastes chrysomelas/carnatus*</u> Acclimation Temperature: 16.0C

$\overline{X}$ Standard Length: 51.1 mm.
$\overline{X}$ Weight: 3.7 g.

<u>Experiment 5e</u> 11-16-78 through 11-20-78

$\overline{X}$ Experiment Temperatures, C:	16.0	21.9	24.1	25.0	25.9	27.4
Standard Deviations:	0.00	0.19	0.05	0.24	0.38	0.70

<u>Experiment 5g</u> 11-29-78 through 12-03-78

$\overline{X}$ Experiment Temperatures, C:	16.0	22.9	23.5	24.0	24.5	24.9
Standard Deviations:	0.02	0.05	0.06	0.05	0.03	0.09

<u>Zero Mortality, C:</u> 16.0, 21.9

PERCENTAGE MORTALITY

Experiment Code	5g	5g	5g	5e	5g	5g	5e	5e	5e
Temperature, C	22.9	23.5	24.0	24.1	24.5	24.9	25.0	25.9	27.4
Time (hours) 1									100
2								20	
3					10	10		50	
4							10	70	
5						20	40	90	
6							50	100	
7					20	30			
8		10				60			
9									
10						80			
11						90			
12		20			30	100			
18	10		20	10	40		100		
30					50				
42									
54				20					
66				30					
78					60				
90					70				
96									
Total Percentage Mortality	10	20	20	30	70	100	100	100	100

16.0C ACCLIMATION TEMPERATURE

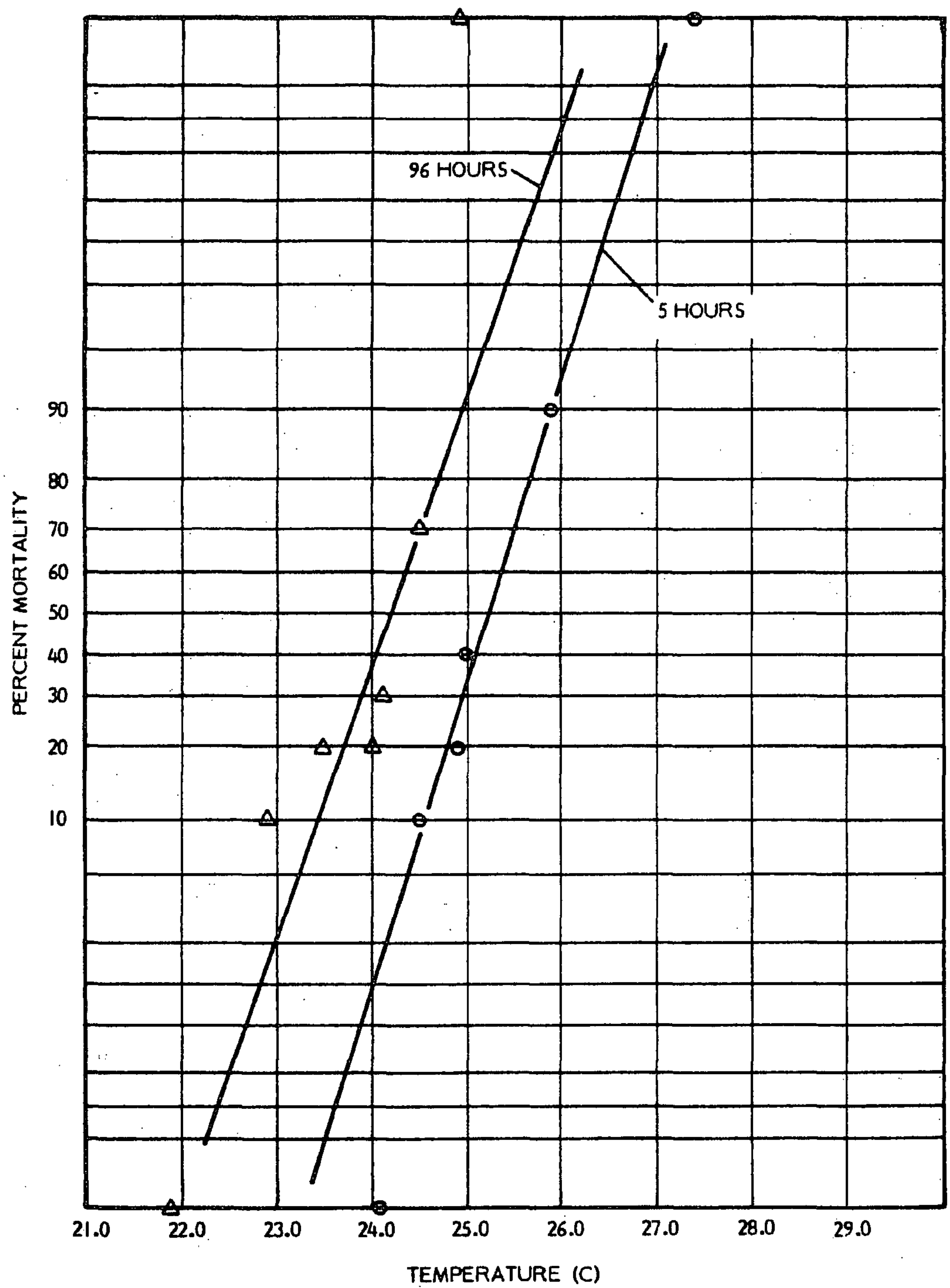

FIGURE 2.1-1

PERCENTAGE THERMAL MORTALITY VERSUS TEMPERATURE AT 7, 8 AND 9 HOURS FOR JUVENILE BLACK-AND-YELLOW/GOPHER ROCKFISH ACCLIMATED TO 12.2 C IN EXPERIMENTS 5e and g.

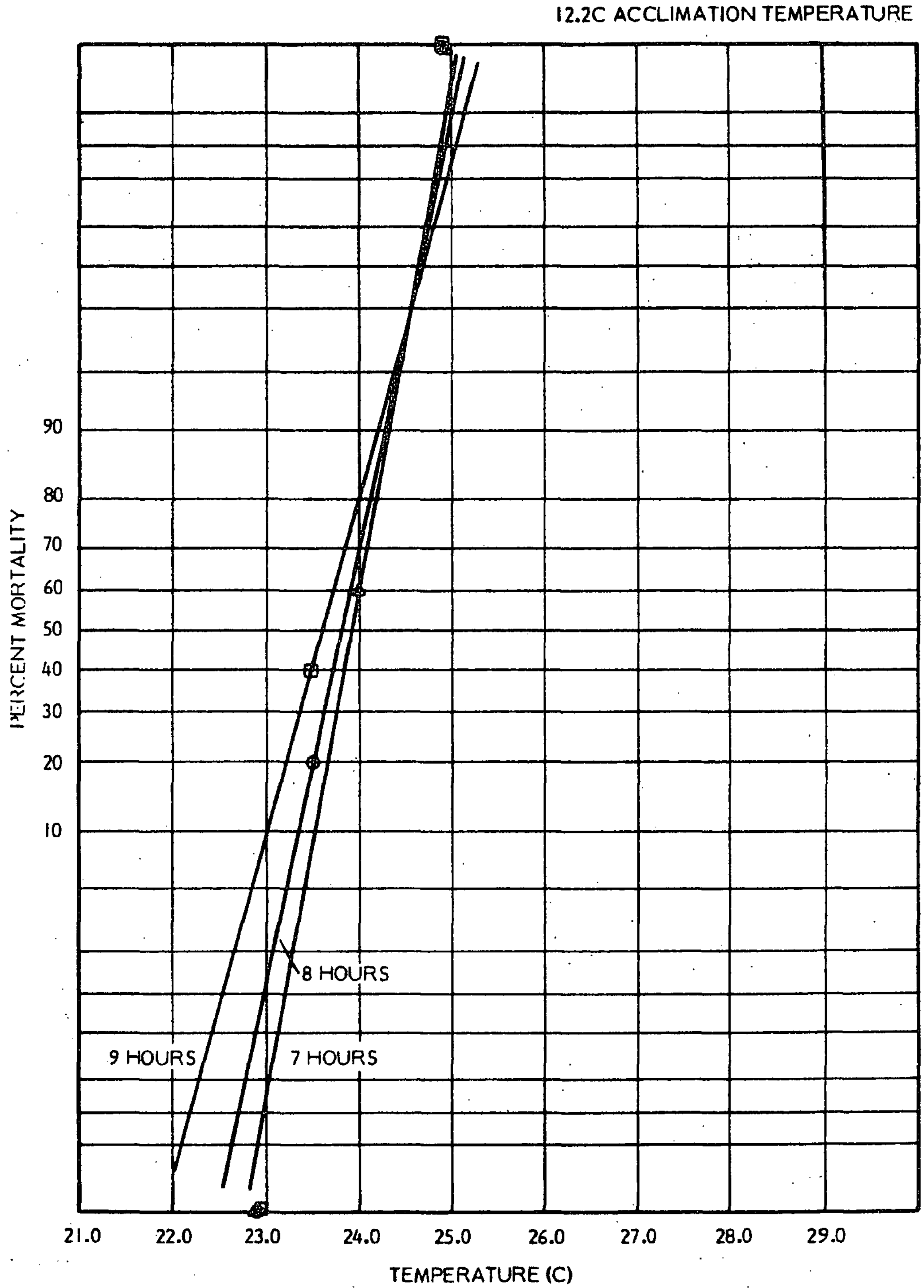

FIGURE 2.1-2

PERCENTAGE THERMAL MORTALITY VERSUS TEMPERATURE AT 7, 8 AND 9 HOURS FOR JUVENILE BLACK-AND-YELLOW/GOPHER ROCKFISH ACCLIMATED TO 12.2 C IN EXPERIMENTS 5f and h.

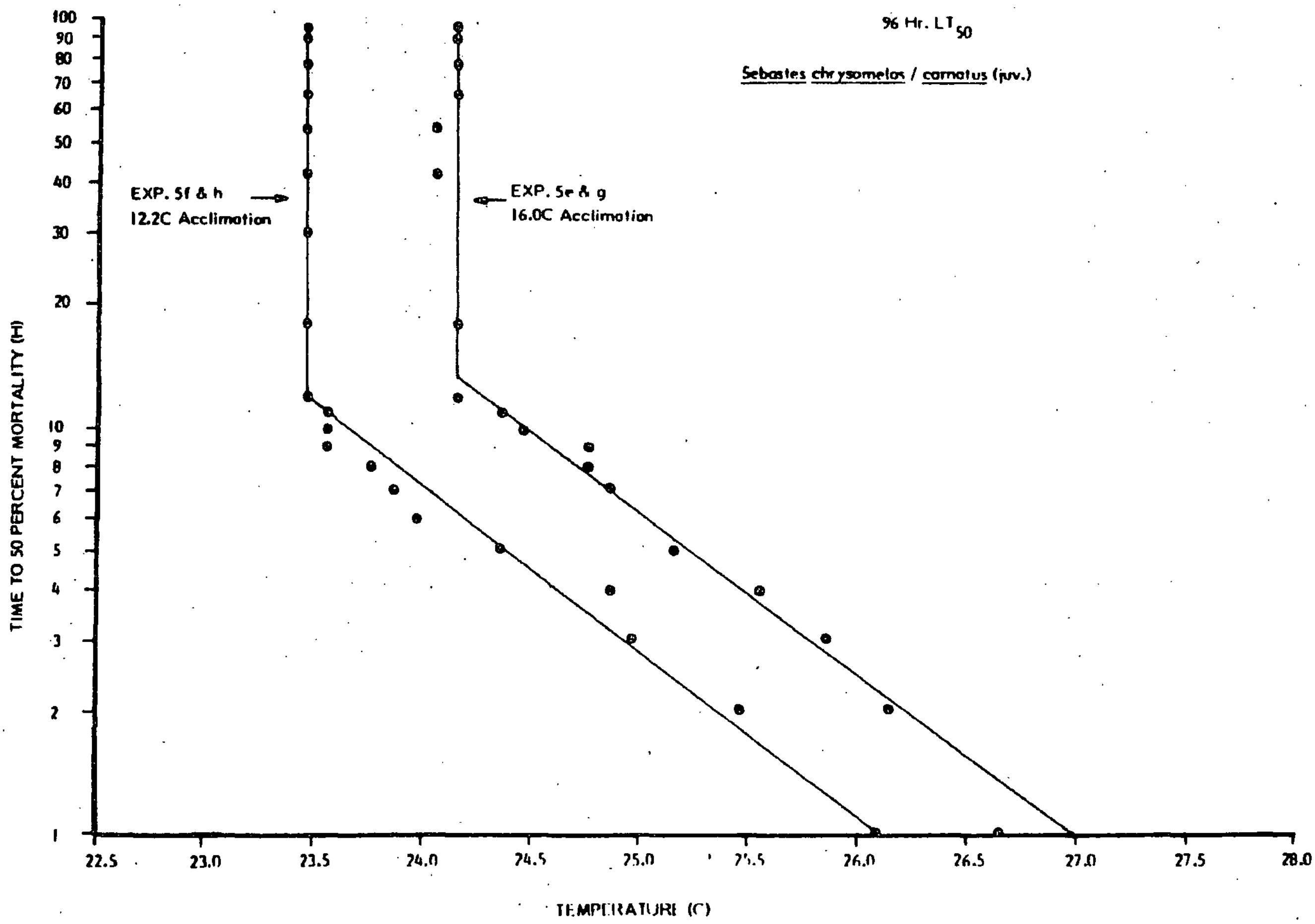

FIGURE 2.1-3

TIME TO 50 PERCENT THERMAL MORTALITY VERSUS TEMPERATURE OF JUVENILE BLACK-AND-YELLOW/GOPHER ROCKFISH ACCLIMATED TO 12.2 C (EXPERIMENTS 5f AND h) AND 16 C (EXPERIMENTS 5e AND g)

50 percent mortality (LT50) occurs for any length of exposure up to 96 hours can be estimated graphically from the hand-plotted curves for each group. The 96-hour LT50 for juvenile black-and-yellow/gopher rockfish acclimated to 16.0C was 24.2C, and the 96-hour LT50 for those acclimated to 12.2C was 23.5C. Note that for the 12.2C acclimated group, exposures to 23.5C for periods longer than about 12 hours (50 percent mortality exposure) did not produce any additional mortality. For the 16C acclimated group, exposures to 24.2C for periods longer than about 12 hours (50 percent mortality exposure) did not produce any additional mortality. Therefore, 23.5C and 24.2C are the estimated "upper incipient lethal temperature" for the 12.2C and 16C acclimation groups, respectively.

2.1.1.3 DISCUSSION

Juvenile black-and-yellow/gopher rockfish acclimated to 16C tolerated higher temperatures for longer periods than juveniles acclimated to 12.2C (54F). The warm acclimated group had 96-hour LT50 of 24.2C (75.6F), and the 96-hour LT50 of the cold acclimated group was 23.5C (74.3F). Therefore, these juvenile rockfish would be expected to tolerate elevated temperatures better during warm fall periods than during colder summer periods. Temperatures lower than 23.5C (74.3F) (the "upper incipient lethal temperature") would result in less than 50 percent mortality for the cold acclimated fish, and temperatures less than 24.2C (75.6F) (the "upper incipient lethal temperature") would result in less than 50 percent mortality for the warm acclimated fish.

2.1.2 CRITICAL THERMAL MAXIMUM (CTM)

The objective of the critical thermal maximum experiment was to provide information on the point of mortality of fishes exposed to a rapidly increasing temperature. This standard test provides an index to a species' heat tolerance and can also be used to determine at what point black-and-yellow/gopher rockfish become acclimated to certain temperatures, in preparation for other experiments. The purpose was to provide information about the possible impact of the thermal plume on the species, in particular, the mortality expected when the black-and-yellow/gopher rockfish suddenly encounters relatively high temperatures from which it cannot escape.

2.1.2.1 METHODS

A summary of the methods employed in the critical thermal maximum experiment on black-and-yellow/gopher rockfish is presented below. The protocol for this experiment is included in APPENDIX C of this report (experiments 8o, p, r, and s). A more detailed account of the experimental procedure used in critical thermal maximum experiments is presented in APPENDIX B (Procedure 308).

The juvenile specimens of black-and-yellow/gopher rockfish used in this experiment were collected in July and August 1978 at Morro Bay, north of Diablo Cove, by otter trawl.

The fish were held in 4 x 8 ft flow-through tanks. During the holding period, the fish were carefully observed and those which appeared to be injured or otherwise unhealthy were excluded from use in the experiment.

Temperatures in the tanks were controlled (where required) and recorded by an Autodata Nine computer. The temperature data were transcribed from paper tapes onto data sheets and summarized as cumulative means and standard deviations for 24-hr intervals during the holding period.

The basic procedure of the experiment involved placing the test population into a 60-liter glass aquarium insulated with 2-cm-thick styrofoam sheets on all four vertical sides. Observation windows were cut into the sheets on two sides and the top was left uncovered. All portions of the aquarium were thus easily visible. The seawater was heated by a single 500W immersion heater and the temperature increase (0.1 C/min) was manually controlled through a variable voltage regulator. Airstones positioned at the bottom of the tank provided a continuous stream of bubbles, which maintained high oxygen levels and prevented thermal stratification. Temperature was recorded using a single YSI model 401 thermistor probe connected to the Autodata Nine computer. Immediately prior to each run, the test tank was filled with seawater at the holding temperature of the test population. The fish were then transferred to the test tank and allowed to adjust to the new surroundings for 15-60 min.

After the adjustment period, heating of the water commenced at the rate of 0.1 C/min. The fish were initially observed at 15-min intervals. The temperature of the tank and comments relating to the condition of the fish were recorded at each observation. As the fish appeared to show signs of severe stress (disorientation and/or loss of equilibrium, depending on the species), observations were made continuously and upon the death of each individual (defined by cessation of opercular movement) the time, number of deaths and temperature of the water were recorded until all of the fish in a test population had succumbed. The mean value of the temperatures recorded at death represented the critical thermal maximum value for that test.

The experiment was used to test populations of black-and-yellow/gopher rockfish that were acclimating to a constant controlled temperature.

A one-way ANOVA test was used to detect significant differences between runs. If significant differences were detected, a posteriori comparisons were performed using Duncan's new multiple range test to determine which of the runs differed.

2.1.2.2 RESULTS

A total of four CTM experiments, comprising four runs each, were conducted on this species. Each run involved four fish. The runs were made on fish acclimating to controlled temperatures. Summary descriptions of the experiments follow:

- experiment 8o: first run 15 September 1978, acclimating to 16C (raised from ambient, approximately 14C, at 1C/day), runs after 1, 3, 4, and 5 days at 16C

- o <u>experiment 8p</u>: first run 22 September 1978, acclimating to 12C (raised from ambient, approximately 13C, at 1C/day), runs after 5, 8, 9, and 10 days at 12C

- o <u>experiment 8r</u>: first run 6 November 1978, acclimating to 12C (raised from ambient, approximately 13C, at 1C/day), runs after 3, 4, 5, and 11 days at 12C

- o <u>experiment 8s</u>: first run 8 November 1978, acclimating to 16C (raised from ambient, approximately 14C, at 1C/day), runs after 0, 1, 2, and 5 days at 16C.

Mean CTM values for black-and-yellow/gopher rockfish in these tests ranged from 18.4 to 19.5C. The data and results of statistical tests from all of these runs are presented in TABLES 2.1-3 and 2.1-4.

2.1.2.3 DISCUSSION

A significant although slight increase in CTM value was detected in one experiment where black-and-yellow/gopher rockfish were held at 16C (61F) for five days. Acclimation of black-and-yellow/gopher rockfish probably occurred after five days at this constant temperature. No significant differences were detected in populations of black-and-yellow/gopher rockfish held at 12C (54F) for up to 11 days. This is not surprising in view of the fact that the ambient temperatures at which the fish were held during the period preceding acclimation were only about 1 Celsius degree (1.8 Fahrenheit degrees) higher than the acclimation temperature.

TABLE 2.1-3

SUMMARY DATA: BLACK-AND-YELLOW/GOPHER ROCKFISH CRITICAL THERMAL MAXIMUM EXPERIMENTS 8O, 8P, 8R, AND 8S

Experiment (Holding Temperature (C))	Run	Mean Length (mm)	Mean Weight (g)	No. Fish	Mean CTM	Days at Holding Temperature
8o (16)	1	31	0.7	4	28.7	1
	2	34	1.1	4	29.2	3
	3	32	0.9	4	29.4	4
	4	35	1.3	4	29.5	5
8p (12)	1	39	1.4	4	28.9	5
	2	38	1.7	4	28.6	8
	3	41	1.7	4	28.4	9
	4	42	2.0	4	28.7	10
8r (12)	1	46	2.7	4	28.9	3
	2	46	3.0	4	28.6	4
	3	46	2.6	4	28.8	5
	4	52	3.5	4	28.4	11
8s (16)	1	47	2.9	4	29.1	0
	2	46	2.7	4	28.8	1
	3	49	3.3	4	28.9	2
	4	49	3.3	4	28.8	5

TABLE 2.1-4

STATISTICAL RESULTS: BLACK-AND-YELLOW/GOPHER ROCKFISH CRITICAL THERMAL MAXIMUM EXPERIMENTS 8O, 8P, 8R, AND 8S

Test	Experiment	Hold. Temp. (C)	Result
ANOVA:	8o	16	$F_{(3,12)} = 17.831$ (significant at 0.01)
	8p	12	$F_{(3,12)} = 3.010$ (not significant at 0.01)
	8r	12	$F_{(3,12)} = 1.389$ (not significant at 0.01)
	8s	16	$F_{(3,12)} = 0.6272$ (not significant at 0.01)

Duncan's Test (bars connect runs not significantly different)[a]

Days at Holding Temperature (16C)	1	2	3	4
CTM (C)	28.7	29.2	29.4	29.5

[a] Only experiment 8o could be analyzed with Duncan's Test because of the lack of statistical significance and large degree of variability in the data of the other experiments.

2.2 BLUE ROCKFISH (SEBASTES MYSTINUS)

According to Miller and Lea (1972), the blue rockfish, *Sebastes mystinus*, occurs in coastal waters from the surface to 300 ft (100 m) depth. Its geographical distribution extends from the Bering Sea to Punta Banda, Baja California, Mexico (Miller and Lea 1972, 1976). It reaches a maximum length of 53 cm (21 in.). The blue rockfish is an important species in the ecology of Diablo Cove. The literature on blue rockfish was reviewed in a previous report (PGandE 1979b).

Two types of heat tolerance experiments were conducted on blue rockfish juveniles. 96-hour median lethal (LT50) tests and critical thermal maximum (CTM) tests were conducted on 12 and 16 C-acclimated animals.

2.2.1 96-HOUR MEDIAN LETHAL TEMPERATURE (96-H LT50)

The study objectives of the LT50 experiment on juvenile blue rockfish (*Sebastes mystinus*) were the following:

- To observe the time course of mortality for groups of fish exposed to heated water over a 96-hour period
- To determine from these observations the temperatures at which 50 percent of the fish die (LT50 values) for standardized 96-hour exposures and for shorter exposures to heated water
- To measure the effect of acclimation to warm (16 C) and cold (12 C) temperatures on the LT50 values for the fish.

The acclimation temperatures were selected to represent average cold season (12 C) and warm season (16 C) water temperatures in the Diablo Canyon area, in order to assess the effect of seasonal ambient water temperatures on the species' thermal tolerance. The purpose of this study was to provide information on the effects of the power plant discharge on juvenile blue rockfish at different times of the year and when acclimated to different temperatures.

2.2.1.1 METHODS

Juvenile blue rockfish were collected using a 12 x 12 ft diver-operated lift net. The collections for experiments 5i, j, k and l were made in the Diablo Canyon power plant intake cove (that area enclosed by the two breakwaters) during January 1979. These fish had a mean standard length of 90 mm and a mean wet weight of 19.0 g (approximately one year old according to Miller and Geibel 1973). Specimens used in experiments 5m and n were collected using the same method from the area just outside the south breakwater. These collections were made in June 1979. The mean standard length for these fish was 64 mm and the mean wet weight was 7.0 g (less than one year old).

Methods used for experiments 5i, j, k and l were identical to those used for black-and-yellow/gopher rockfish (see SECTION 2.1.1.1 of this report). The protocols for these runs are included in APPENDIX C. Experiments 5m and 5n differed slightly from the other experiments in the manner in which the fish were confined. Both experiments were run simultaneously in common 4 x 8 ft tanks. The ten 12 C acclimated fish (experiment 5m) were held in a 2 x 3 ft cage while the ten 16 C acclimated blue rockfish (experiment 5n) shared the remainder of the tank with a like number of 16 C acclimated olive rockfish (Sebastes serranoides) (experiment 5o). Animal density was sufficiently low that no overcrowding occurred and no agonistic interactions were observed between the two species (in nature, juveniles of these species are commonly observed schooling together). The temperature records and methods of data analysis were the same as those used in earlier experiments. Experiments 5i, j, k and l represent two pairs of runs, each pair consisting of a 12 C and 16 C acclimation group. Experiments 5i (16 C) and 5j (12 C) began on 12 February 1979; experiments 5k (16 C) and 5l (12 C) began on 22 February 1979; and experiments 5m and 5n began on 13 August 1979.

2.2.1.2 RESULTS

Juvenile blue rockfish acclimated to 12 C and 16 C were tested at a variety of temperatures ranging from 19.9 to 25.9 C. The time courses of mortality for these experiments are shown in TABLES 2.2-1 through 2.2-4. Data from those experiments run in February 1979 (5i, j, k and l) were grouped by acclimation temperature (12 C and 16 C) and appear in TABLES 2.2-1 and 2.2-3, respectively. Data from experiments 5m and 5n (August 1979) were kept separate from the above because of differences in season, specimen size and age class: these data appear in TABLES 2.2-2 and 2.2-4, respectively. For each observation, cumulative mortalities were plotted on a probability scale versus their respective mean test temperatures. Example plots are shown in FIGURE 2.2-1 of the 6- and 18-hour observations of the 16 C acclimated fish (experiments 5i and 5k), and in FIGURE 2.2-2 for the 6- and 18-hour observations of the 12 C acclimated fish (experiments 5j and 5l). A line fitted by eye to each of the four plots was used to extrapolate the temperature at which exactly 50 percent mortality of each group of fish would occur. In each acclimation group, the extrapolated temperatures which caused 50 percent mortality were then plotted for each observation period through 96 hours (FIGURES 2.2-3 and 2.2-4). The temperature at which 50 percent mortality (LT50) occurs for any length of exposure up to 96 hours can be estimated graphically from the hand-plotted curves for each group.

The 96-hour LT50 for juvenile blue rockfish acclimated to 16 C was 23.1 C for those fish tested in experiments 5i and 5k and 23.2 C for those in experiment 5n. The 96-hour LT50 for the same species acclimated to 12 C was 22.9 C (experiments 5j and 5l) and 22.2 C (experiment 5m).

2.2.1.3 DISCUSSION

In reviewing the data from experiments 5i-n certain points should be noted with respect to the thermal tolerance of juvenile blue rockfish:

1. In both the February and August experiments there was an increase in thermal tolerance in the 16 C (61 F) as compared to the 12 C (54 F) acclimated fish (FIGURES 2.2-3 and 2.2-4). An example of this increase is shown in TABLE 2.2-5.

2. Thermal tolerance was also found to increase with increasing size of fish.

TABLE 2.2-1

BLUE ROCKFISH HEAT TOLERANCE:

SUMMARY TEMPERATURE AND THERMAL MORTALITY DATA FOR TWO EXPERIMENTS ON 12C ACCLIMATED ANIMALS IN FEBRUARY 1979

Mean Standard Length: 91 mm
Mean Wet Weight: 19.0 g

Experiment 5j: 02-12-79 through 02-16-79						
Mean Experimental Temperatures (C):	12.0	20.0	22.1	24.1	24.8	25.8
Standard Deviations:	0.07	0.03	0.04	0.05	0.07	0.00
Experiment 5l: 02-22-79 through 02-26-79						
Mean Experimental Temperatures (C):	12.0	22.0	22.6	23.1	23.5	24.0
Standard Deviations:	0.04	0.05	0.05	0.04	0.06	0.15
Zero Mortality (C):	12.0	20.0	22.1			

96H CUMULATIVE PERCENTAGE MORTALITY

Experiment Code:	5l	5l	5l	5l	5l	5j	5j	5j
Temperature (C):	22.0	22.6	23.1	23.5	24.0	24.1	24.8	25.8
Hours								
1				30	40	30	50	100
2			10	40	70		100	
3		10		50	80	40		
4		20			100	50		
5								
6				60		90		
18	10	30	20	100		100		
30			30					
90		40						
96	10	40	40	100	100	100	100	100

TABLE 2.2-2

BLUE ROCKFISH HEAT TOLERANCE:

SUMMARY TEMPERATURE AND THERMAL MORTALITY DATA FOR ONE EXPERIMENT ON 12C ACCLIMATED ANIMALS IN AUGUST 1979

Mean Standard Length: 64 mm
Mean Wet Weight: 6.6 g

Experiment 5m: 08-13-79 through 08-17-79

Mean Experimental Temperatures (C):	12.1	20.0	21.9	23.1	23.8	25.0	25.6*
Standard Deviation:	.07	.05	.04	.01	.09	.05	0.0

Zero Mortality (C): 12.1, 20.0

96 H CUMULATIVE PERCENTAGE MORTALITY

Temperature (C):	21.9	23.1	23.8	25.0	25.6
Hours					
1		20	50	80	100
2	30	40	70	90	
3			80	100	
4		90			
5					
6					
18		50	100		
30		60			
42	40				
54		70			
66					
78		90			
90					
96	40	90	100	100	100

*One reading only.

TABLE 2.2-3

BLUE ROCKFISH HEAT TOLERANCE:

SUMMARY TEMPERATURE AND THERMAL MORTALITY DATA FOR TWO EXPERIMENTS ON 16 C ACCLIMATED ANIMALS IN FEBRUARY 1979

Mean Standard Length: 90 mm
Mean Wet Weight: 18.9 g

Experiment 5i: 02-12-79 through 02-16-79

Mean Experimental Temperatures (C):	16.0	19.9	22.0	24.1	25.0	25.9
Standard Deviations:	0.04	0.05	0.05	0.05	0.02	0.08

Experiment 5k: 02-22-79 through 02-26-79

Mean Experimental Temperatures (C):	16.0	21.9	22.5	23.1	23.5	24.0
Standard Deviations:	0.05	0.07	0.05	0.04	0.02	0.06

Zero Mortality (C): 16.0, 19.9

96H CUMULATIVE PERCENTAGE MORTALITY

Experiment Code	5k	5i	5k	5k	5k	5k	5i	5i	5i
Temperature (C)	21.9	22.0	22.5	23.1	23.5	24.0	24.1	25.0	25.9
Hours									
1									10
2									80
3									90
4								20	
5									
6								30	100
18			10			20	20	100	
30				10		40			
42					10	80	80		
54					20	100	100		
66			20		50				
78				20	90				
90		10		30	100				
96	10	10	20	30	100	100	100	100	100

TABLE 2.2-4

BLUE ROCKFISH HEAT TOLERANCE:

SUMMARY TEMPERATURE AND THERMAL MORTALITY DATA FOR ONE EXPERIMENT ON 16C ACCLIMATED ANIMALS IN AUGUST 1979

Mean Standard Length: 65 mm
Mean Wet Weight: 7.3 g

Experiment 5n: 08-13-79 through 08-17-79

Mean Experimental Temperatures (C):	16.0	20.0	21.9	23.1	23.8	25.0	25.8
Standard Deviations:	.05	.05	.04	.01	.07	.05	0.0

Zero Mortality (C): 16.0, 20.0, 21.9*

96 H CUMULATIVE PERCENTAGE MORTALITY

Temperature (C):	23.1	23.8	25.0	25.8
Hours				
1			40	50
2			50	100
3				
4			60	
5			80	
6				
18		20	100	
30		40		
42		100		
54	10			
66	20			
78				
90				
96	20	100	100	100

* One fish became trapped beneath the 2 x 3-ft cage and died as a result; this was not a thermal death.

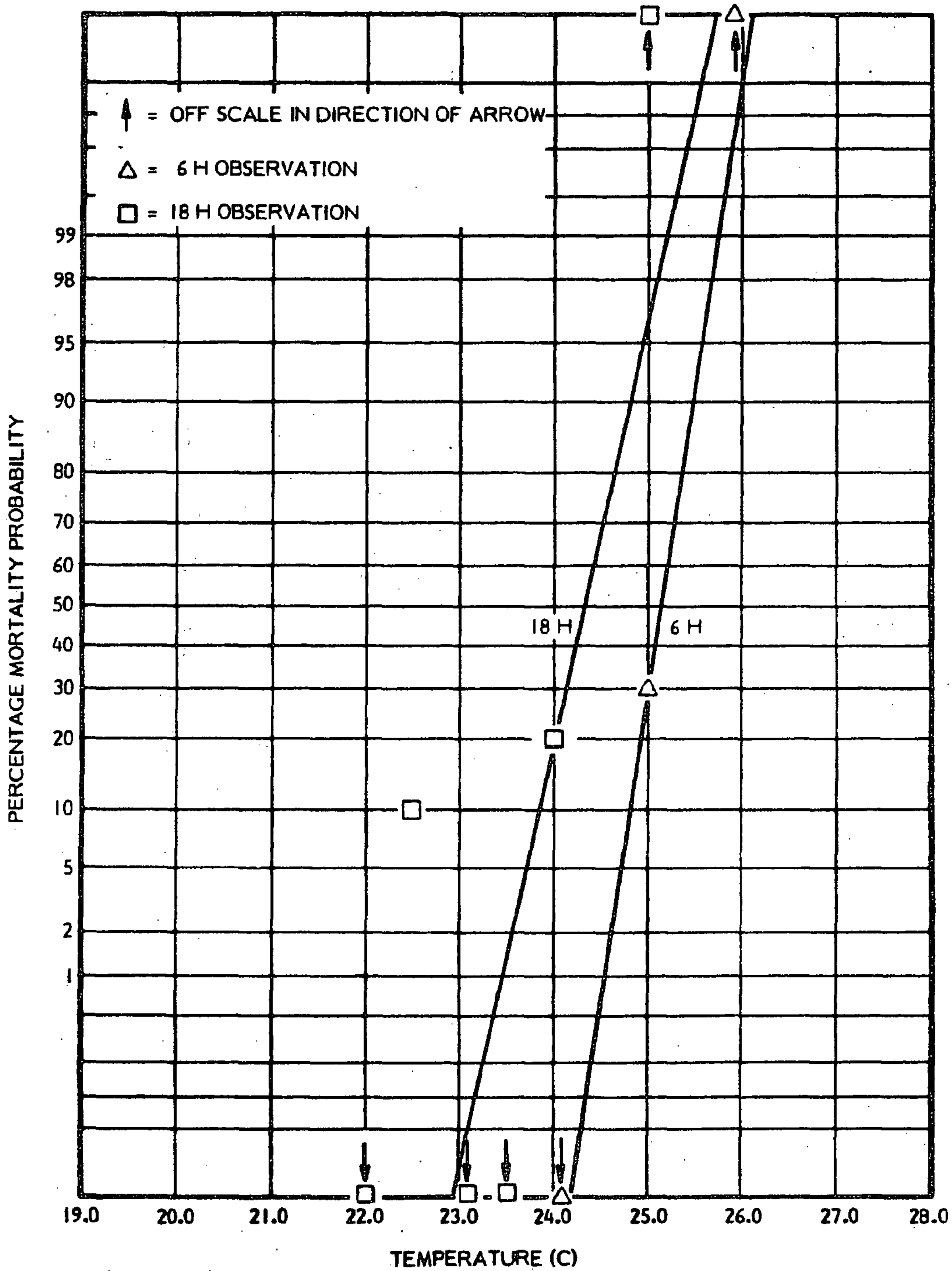

FIGURE 2.2-1

BLUE ROCKFISH HEAT TOLERANCE:

PERCENTAGE MORTALITY PROBABILITY VERSUS
TEMPERATURE AT 6 AND 18 HOURS

The animals were acclimated to 16C
(Experiments 5i and 5k)

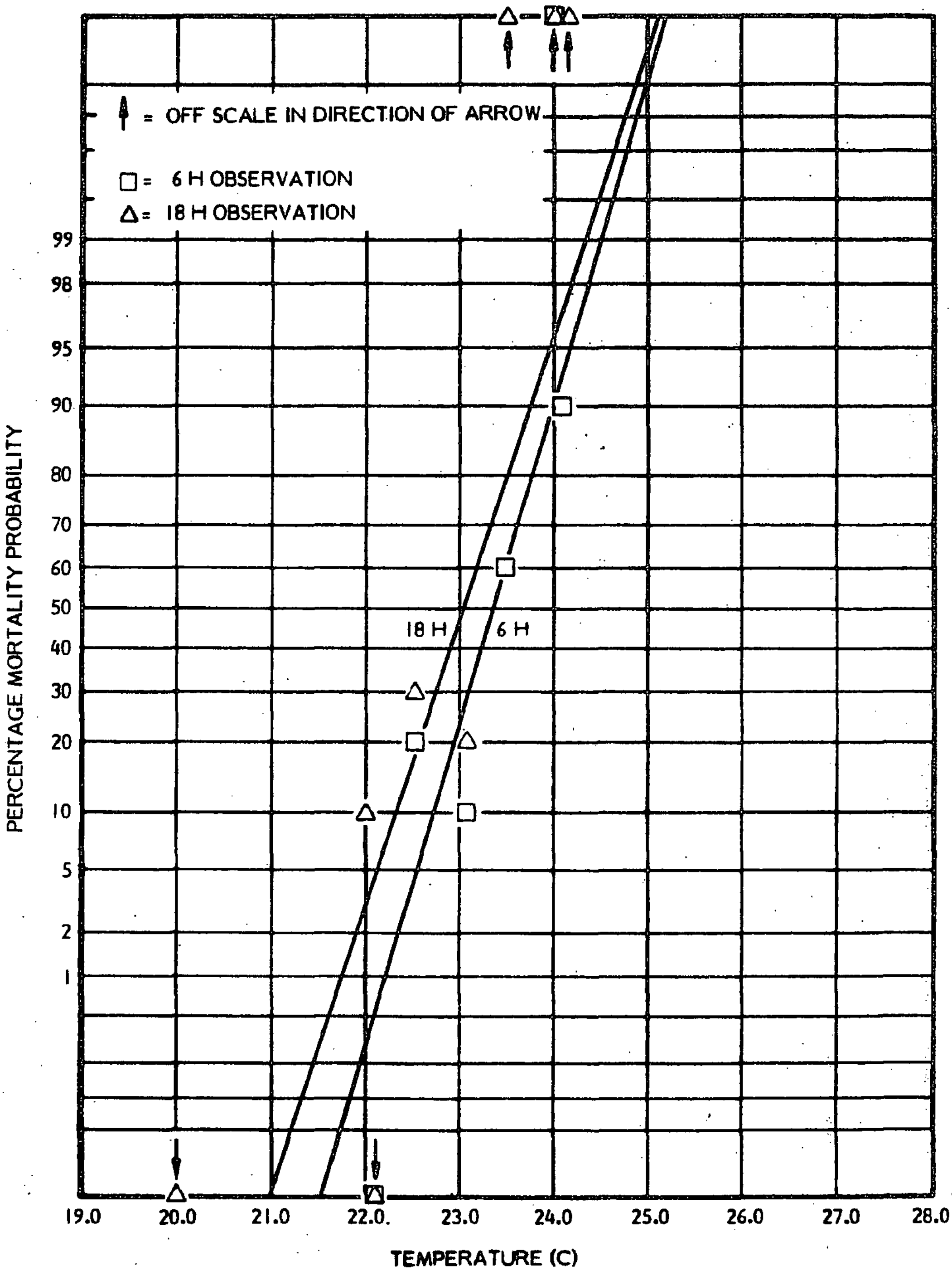

FIGURE 2.2-2

BLUE ROCKFISH HEAT TOLERANCE:

PERCENTAGE MORTALITY PROBABILITY VERSUS TEMPERATURE AT 6 AND 18 HOURS

The animals were acclimated to 12C (Experiments 5j and 5l)

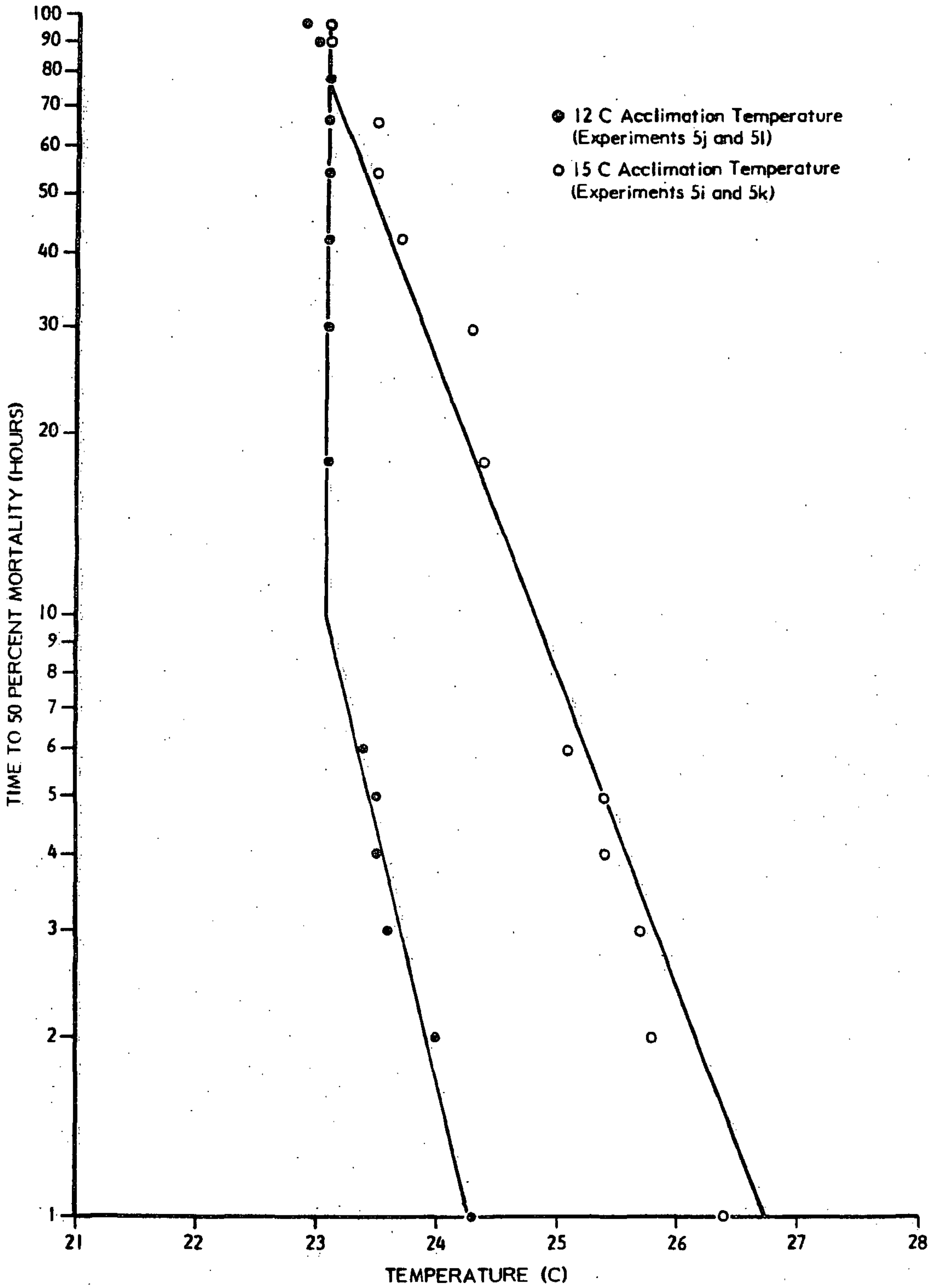

FIGURE 2.2-3

BLUE ROCKFISH HEAT TOLERANCE:
TIME TO 50 PERCENT MORTALITY
IN FEBRUARY 1979 EXPERIMENTS

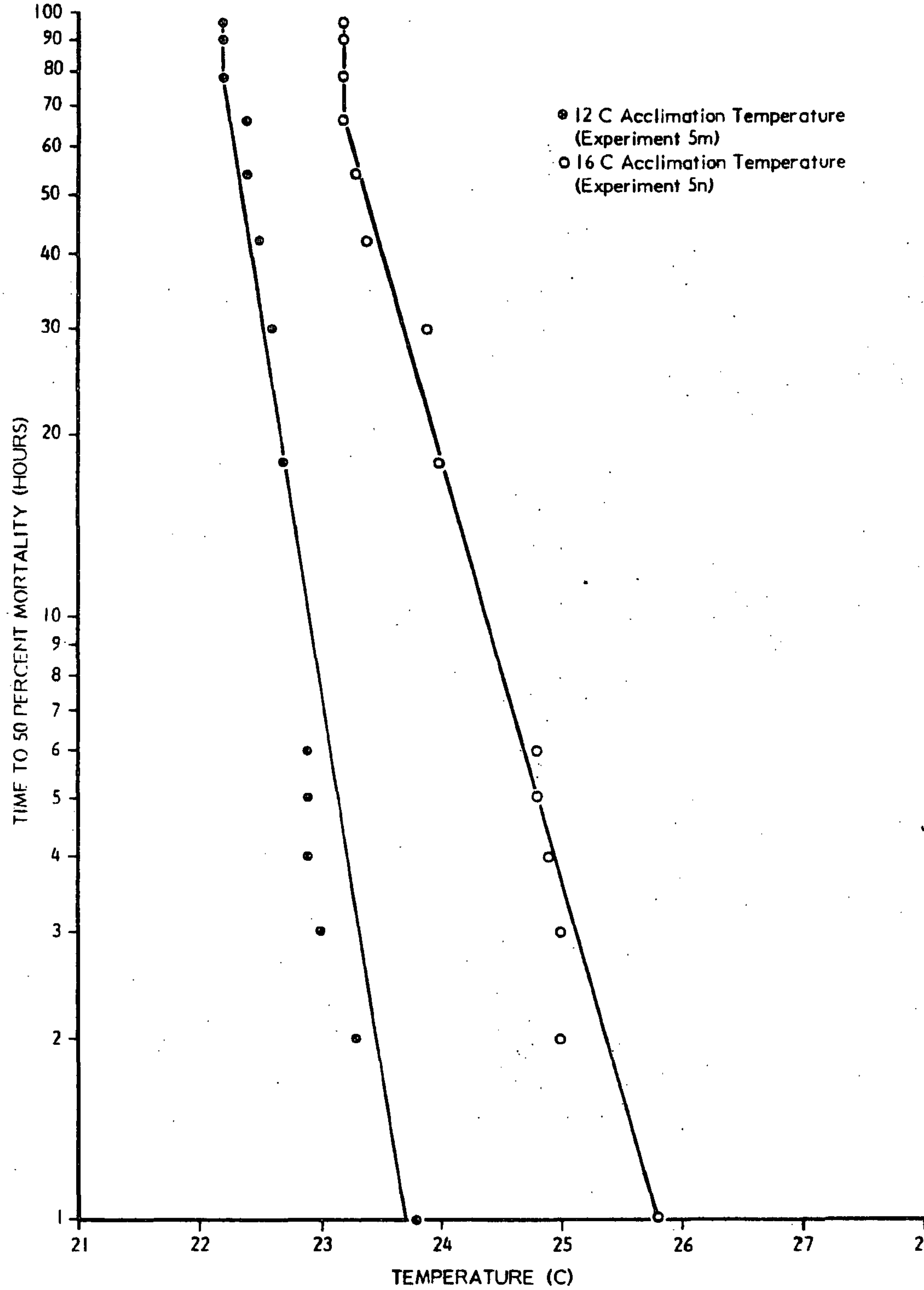

FIGURE 2.2-4

BLUE ROCKFISH HEAT TOLERANCE:
TIME TO 50 PERCENT MORTALITY
IN AUGUST 1979 EXPERIMENTS

TABLE 2.2-5

BLUE ROCKFISH HEAT TOLERANCE:
COMPARISON OF TIMES TO 50 PERCENT MORTALITY
FOR ANIMALS TESTED AT DIFFERENT SEASONS

Experiment Code	Experiment Date (1979)	Acclimation Temperature (C)	Mean Standard Length (mm)	Mean Wet Wt (g)	Time (h) to 50% Mortality at 23.5 C
5j and l	February	12	91	19.0	5
5m	August	12	64	6.6	2
5i and k	February	16	90	18.9	49
5n	August	16	65	7.3	41

From the experiments conducted it cannot be determined what part, if any, of this increase is attributable to the different year classes to which the February and August populations belong.

Seasonal changes in thermal tolerance may also have occurred between the winter and summer experiments. Classically, however, thermal tolerance would be expected to increase during the warm water months (Fry 1967). If such an increase does occur for blue rockfish, then the actual differences due to size and/or year class would be even more pronounced than those shown in the above table.

No mortality of cold season (12 C = 54 F) acclimated blue rockfish would be expected below 20.0-22.1 C (68.0-71.8 F) for exposures up to 96 hours. About one half of these fish would be expected to die at 22.2-22.9 C (72.0-73.2 F) after a 96-hour exposure. Of warm season acclimated (16 C = 61 F) blue rockfish, no mortality would be expected below 19.9-21.9 C (67.8-71.4 F) and about one half of these fish would be expected to die at 23.1-23.2 C (73.6-73.8 F) for exposures of 96 hours.

2.2.2 CRITICAL THERMAL MAXIMUM (CTM)

The objective of the critical thermal maximum experiment was to provide information on the point of mortality of fishes exposed to a rapidly increasing temperature. This standard test provides an index to a species' heat tolerance and can also be used to determine at what point blue rockfish become acclimated to certain temperatures, in preparation for other experiments. The purpose was to provide information about the possible impact of the thermal plume on the species, in particular, the mortality expected when the blue rockfish suddenly encounters relatively high temperatures from which it cannot escape.

2.2.2.1 METHODS

A summary of the methods employed in the critical thermal maximum experiment was presented in SECTION 2.1.2.1 of this report. The protocol for this experiment is included in APPENDIX C of this report (experiment 8k). A more detailed account of the experimental Procedure 308 used in critical thermal maximum experiments is presented in APPENDIX B (Procedure 308).

The specimens of blue rockfish used in this experiment were collected during February, March and April 1978 in the vicinity of Diablo Canyon Power Plant by lift net.

One experiment, comprising nine successful runs, was conducted on this species. Each run involved four fish. The runs were made on fish acclimating to 16C.

A one-way ANOVA test was used to detect significant differences between runs. If significant differences were detected, a posteriori comparisons were performed using Duncan's new multiple range test to determine which of the runs differed.

2.2.2.2 RESULTS

One experiment, comprising nine successful runs, was conducted on this species. The data from all of these runs are presented in TABLE 2.2-6.

2.2.2.3 DISCUSSION

Mean CTM values for all runs ranged over 1C (26.4-27.4 C or 79.5-81.3 F) over the 23-day acclimation period. In general, CTM values showed significant increases with increasing acclimation time. CTM values reached 27.4 C (81.3 F) after 16 days of acclimation and did not exhibit any further increases after 23 days of acclimation.

TABLE 2.2-6

SUMMARY DATA AND STATISTICAL RESULTS: BLUE ROCKFISH
CRITICAL THERMAL MAXIMUM EXPERIMENT 8K

SUMMARY DATA

Run	Mean Length (cm)	Mean Weight (g)	No. Fish	Mean CTM (C)	Days at 16C
1	89	16.5	4	26.8	0
2	85	14.3	4	26.4	2
3	90	16.2	4	26.8	4
5[a]	89	16.4	4	27.1	7
6	85	16.8	4	27.0	10
8[b]	94	21.5	4	27.2	14
9	92	18.9	4	27.2	16
10	79	13.7	4	27.4	18
11	87	17.2	4	27.4	23

STATISTICAL RESULTS

ANOVA: $F_{(8,27)} = 2.8482$ (significant at 0.05 level)

Duncan's Test (bars connect runs not significantly different)

CTM	26.4	26.8	26.8	27.0	27.1	27.2	27.4	27.4	27.4
Days at 16C	2	0	4	10	7	14	16	18	23

[a] Run 4 was aborted.

[b] Run 7 was aborted.

NOTE: The fish were held at ambient temperatures (approximately 11C) prior to holding at 16C.

2.4 BLACK ABALONE (HALIOTIS CRACHERODII)

The black abalone (Haliotis cracherodii) is an important species in the ecology of Diablo Cove. The literature on this species was reviewed in PGandE (1979b). Two 96-hour ET50 (median effective temperature) experiments were conducted to determine the heat tolerance of black abalone. The first test incorporated a tidal component by means of which one group of test animals was subjected to alternating exposure to air and water. In the second test, animals which had become acclimated to relatively high temperatures in the growth experiment (see SECTION 3.5.2) were tested to evaluate the effect of long-term acclimation to high temperatures on the ET50 value.

2.4.1 TIDAL/NONTIDAL

Information on the heat tolerance of adult black abalone was obtained from a 96-hour test in which the median effective temperature (ET50), i.e., the temperature at which 50 percent of the test population survived, was determined. Animals acclimated to both 11C and 16C were tested to provide information on seasonal changes in thermal tolerance of black abalone. In this experiment, abalone were either continuously submerged or periodically exposed to air, in order to obtain an understanding of the effects of tidal fluctuations on the thermal tolerance of this intertidal organism.

The study objectives were: (1) to determine the 96-hour ET50 temperature tolerance of black abalone under experimental conditions of constant submergence and simulated tidal exposure and submergence and (2) to measure the effect of acclimation temperatures on the 96-hour ET50 value. In addition, the behavioral responses, spawning frequency, and viability of gametes of black abalone during heat stress were observed. The purpose of this study was to provide ecologically meaningful information about the potential effects of elevated temperatures on the black abalone populations in Diablo Cove.

2.4.1.1 METHODS

A summary description of the laboratory methods employed in the investigations into heat tolerance for black abalone is presented below. For a more detailed description of the laboratory methods see APPENDIX B (Procedure 302 and 304) and protocols see APPENDIX C (Experiments 2a-c and 4a).

Large (10-15 cm shell length) black abalone were collected in the Diablo Canyon area from South Control to Pecho Rock and from North Field's Cove. The specimens were acclimated to either 11.5C or 16C in the thermal effects laboratory for at least one week prior to the start of the experiment. During the experiment, abalone from each of the two acclimation regimes were maintained either continuously submerged or held in a cycle of six hours submergence alternating with six hours exposure to air in the laboratory tidal tanks. The experimental design was composed of four treatment groups: 11C acclimated tidal and non-tidal abalone, and 16C acclimated tidal and non-tidal abalone. There were two phases to the experiment: the first phase during June, 1978, measured the thermal tolerance of 16C non-tidal abalone moving freely on the bottom of the tanks; the second phase during August, 1978, measured the thermal tolerance of 16C tidal, 11C tidal, and 11C non-tidal abalone held in trays to position the animals at a constant level in the simulated tidal cycle.

Abalone were observed at six-hour intervals during a period of 96 hours at test temperatures ranging from 24.7C to 29.4C. Control groups of animals were observed at 11.5C and 16C. Air temperatures encountered by abalone during tidal exposure ranged from 17.5C (11.5C water test tank) to 21.5C (28.5C water test tank), but within a test tank the air temperature did not vary more than 0.4C.

Loss of the ability of an abalone to hold to a surface constituted "ecological death" (effective temperature, ET) during the experiment. Abalone which lost the ability to hold during the second phase of the experiment were returned to 16C water to observe any recovery. Other observations of gape, tentacular response, spawning, turgor, unusual behavior, and further details of the experimental methods are explained in Procedure 304 (see APPENDIX B). Usually 10, and in a few cases 20, abalone were tested in each group. A total of 418 abalone, including control groups, were used in this experiment. Probit analysis of the mortality data was used in accordance with Standard Methods (1975). Other statistical treatments are explained in the Results.

2.4.1.2 RESULTS

The time courses of mortality for the test temperatures during the second phase and for some of the test temperatures during the first phase of the experiment are shown in FIGURE 2.4-1. The response to elevated temperatures is extremely abrupt in that the temperature range from no mortality to 100 percent mortality is only about 1.0C for any experimental group. As might be expected, the precise temperature at which a given response occurred depended upon the experimental group. The results of this experiment indicate that no mortality would be expected at or below 25C and that nearly 100 percent mortality would occur from a 12-18 hour dose of 28.0C water on the most sensitive experimental group (11C-accl, no tide).

The mortality data for each six hour observation period were plotted on a probability versus $\log_{10}$ temperature scale (FIGURE 2.4-2, 24- and 66-hour results). These plots show good co-linearity of points necessary for probit analysis. The only exception to co-linearity occurred in the 16C-acclimated, tide abalone at 27C, which experienced high mortality relative to animals of the same group at other temperatures. Careful observation of the animals at 27C did not reveal any abnormalities. All of the animals at 27C were males (by chance), but a statistical test indicated that here was no significant difference in the temperature response due to sex. Thus, the anomalous response of the 27C animals remains unexplained, and these data were omitted from the probit analysis.

Probit regression lines were fitted by eye to determine the temperature at 50 percent mortality for each of the four experimental conditions at each 6 hour

TIME COURSE OF MORTALITY

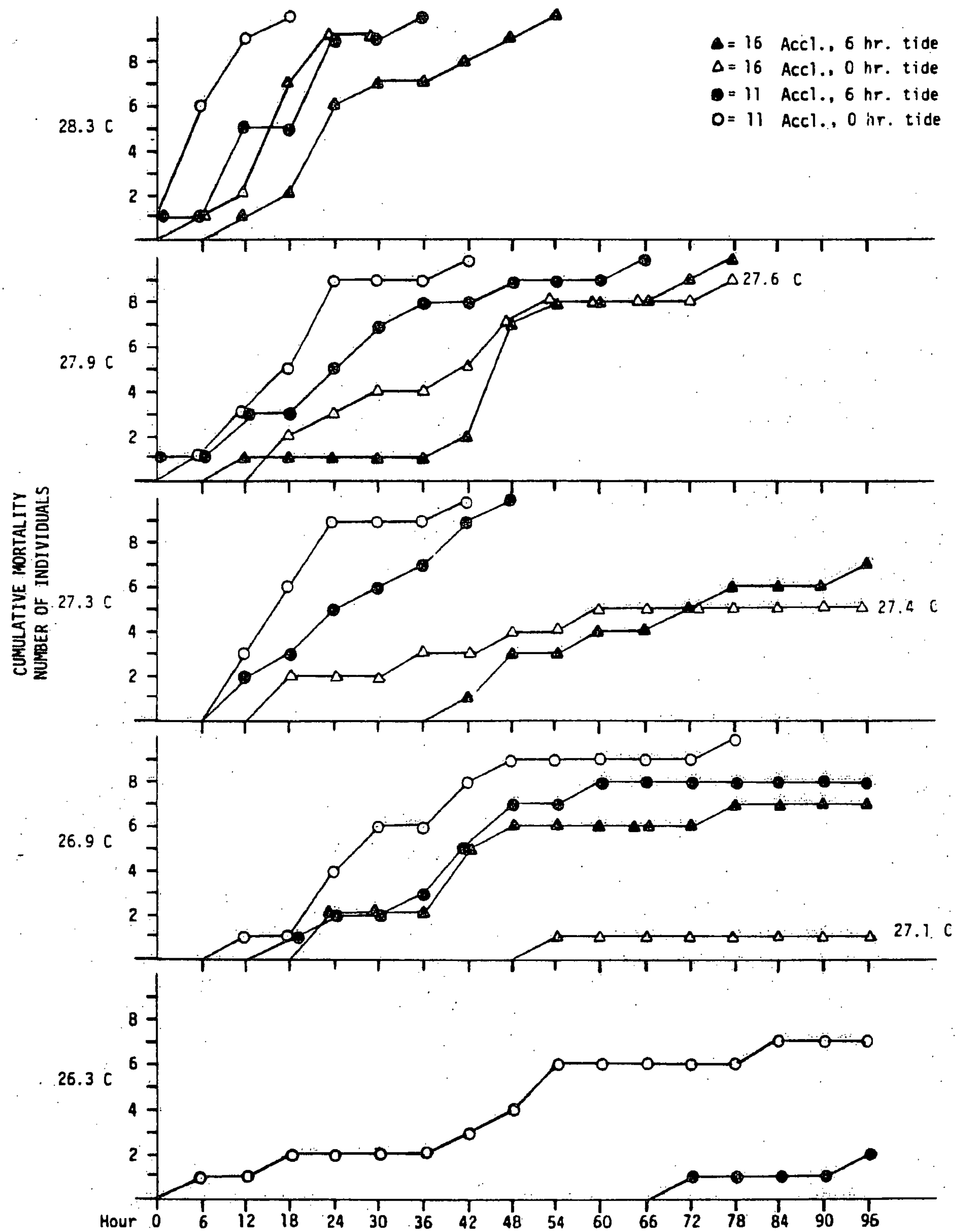

FIGURE 2.4-1

BLACK ABALONE CUMULATIVE MORTALITY VERSUS TIME (96H) AT FIVE TEST TEMPERATURES AND TWO ACCLIMATION TEMPERATURES, WITH AND WITHOUT TIDAL EXPOSURE

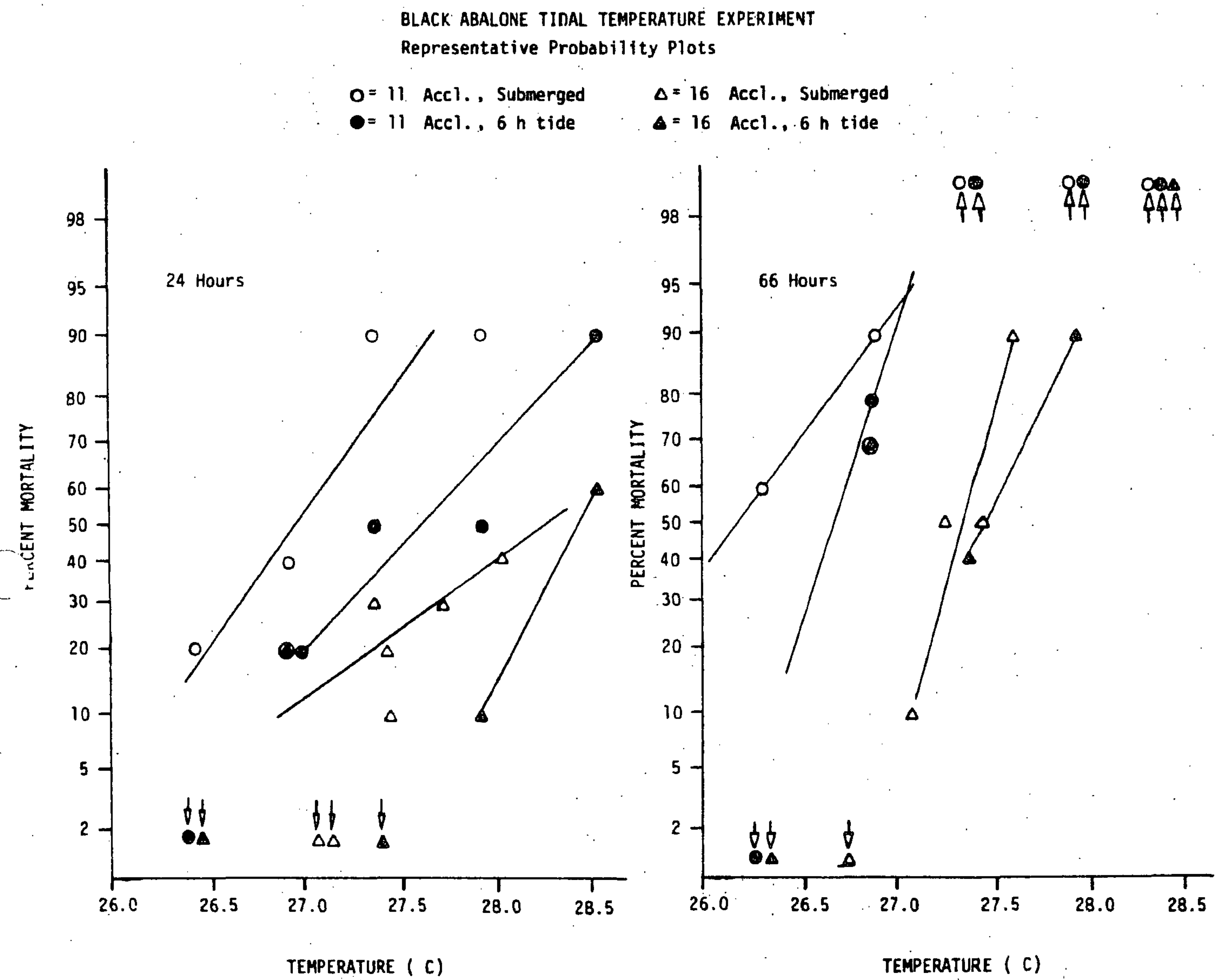

FIGURE 2.4-2

PERCENT MORTALITY PROBABILITY OF BLACK ABALONE AT 24H AND 66H ACCLIMATED TO TWO TEMPERATURES AND EXPOSED TO FIVE TEST TEMPERATURES, WITH AND WITHOUT TIDAL EXPOSURE.

period. For example, water temperatures of 27C produced a 50 percent mortality at 24 hours in the 11C-acclimated, no tide group.

The temperatures at which 50 percent mortality occurred were derived from the probability plots and used to generate the curves of time to 50 percent mortality versus temperature shown in FIGURE 2.4-3. The 11C-acclimated, no tide group had the lowest thermal tolerance (96-hour ET50 = 26.1 C), followed by the 11C-acclimated, tide group (96-hour ET50 = 26.6 C), and by the 16C tide group (96-hour ET50 = 27.2 C). The 16C-acclimated, no tide group was tested under slightly different experimental conditions and earlier in the summer when the abalone were not as sexually ripe. This group had the highest 96-hour ET50 (27.4 C); however, it had a thermal tolerance intermediate between the 11C-acclimated, tide and the 16C-acclimated, tide groups for the test period from 9 to 65 hours. Mortality between the 11C-acclimated, tide, 11C-acclimated, no tide, and 16C-acclimated, tide groups was significantly different (χ^2 = 7.789, 2 d.f.; $p \geq 0.05$). Abalone acclimated to 16C had fewer deaths than those acclimated to 11C (χ^2 = 5.56, 1 d.f.; $p \geq 0.05$), and abalone cyclically exposed, intertidal condition, had fewer deaths than those constantly exposed, subtidal condition (χ^2 = 6.091, 1 d.f.; $p \geq 0.05$) (see TABLES 2.4-1 to 2.4-3).

Males tested in both the first and the second phases of the experiment did not show higher mortality than females (χ^2 = 1.1493 for first phase, χ^2 = 0.4736 for second phase, 1 d.f.; $p \geq 0.10$) (see TABLES 2.4-4 to 2.4-5). Because animals were collected haphazardly with respect to sex, the fact that more males were run in the experiment suggests that the sex ratio of black abalone in the 10-15 cm size range in the Diablo Canyon area is skewed toward males.

The revival rate of abalone that failed to hold a substrate in the first phase of the experiment was not determined but in the second phase of the experiment the revival rate was only 15.8 percent. Most of the abalone that revived were animals which lost the ability to hold during the first 24 hours at temperatures above 27.5C. Abalone that failed to hold later in the test period did not recover.

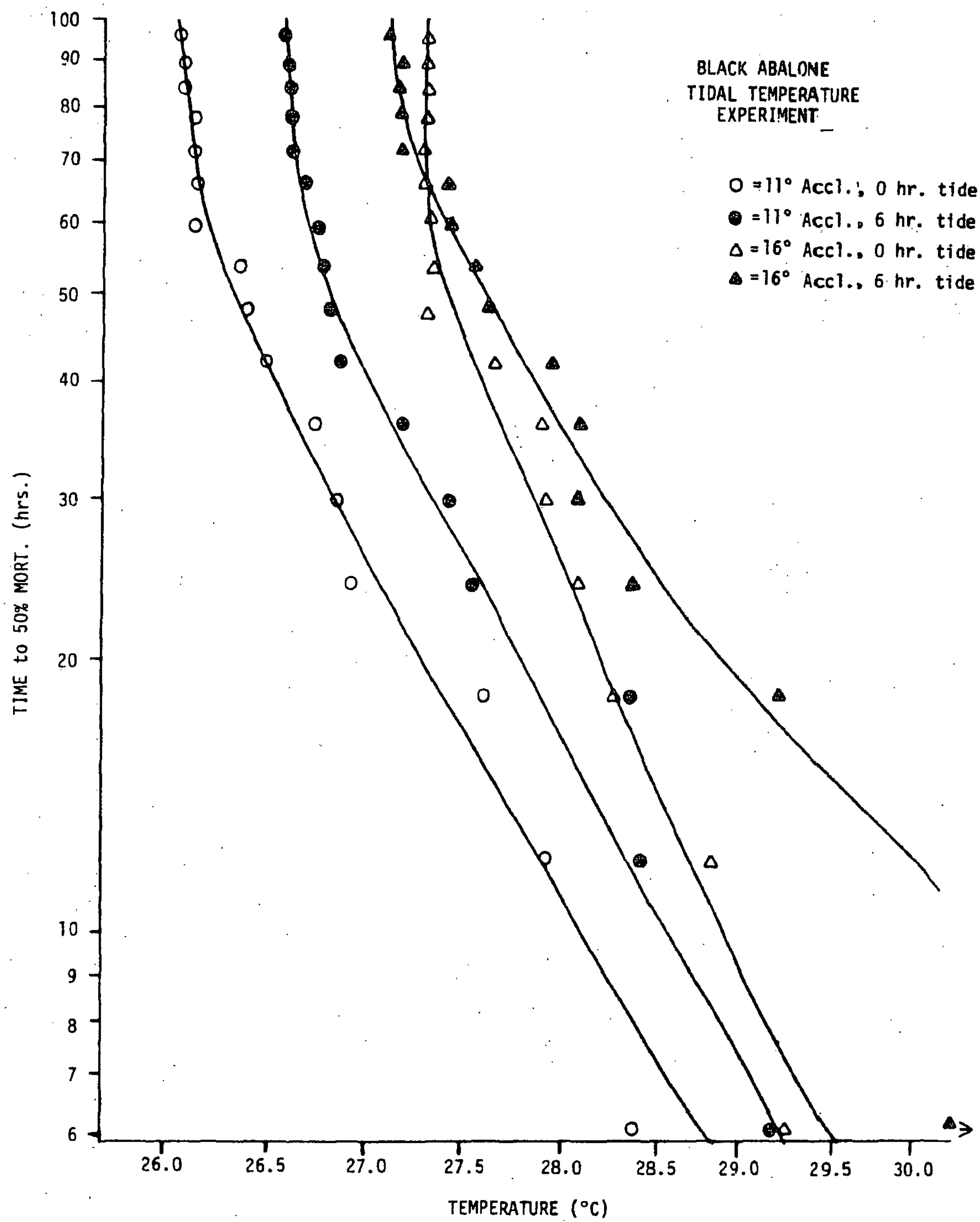

FIGURE 2.4-3

TIME (H) TO 50 PERCENT MORTALITY OF BLACK ABALONE ACCLIMATED TO TWO TEMPERATURES AND EXPOSED TO TEST TEMPERATURES WITH AND WITHOUT TIDAL EXPOSURE.

TABLE 2.4-1

CHI-SQUARE CONTINGENCY TABLE COMPARING MORTALITY OF BLACK ABALONE ACCLIMATED AT 11C AND 16C AND TESTED WITH AND WITHOUT TIDE EXPOSURE AND 11NT GROUPS

The Groups are Signficantly Different

	16C-Accl. Tide	11C-Accl. Tide	11C-Accl. No Tide	Total
Number Dead	34	40	46	120
Number Alive	26	19	11	56
Total	60	59	57	176

$\chi^2 = 7.79$, 2df

$p \geq 0.05$

TABLE 2.4-2

CHI-SQUARE CONTINGENCY TABLE COMPARING CONTINUOUS SUBMERSION AND SIMULATED TIDES ON BLACK ABALONE THERMAL MORTALITY

Thermal Mortalities Were Significantly Different Among Tide/No Tide Groups

	Tide	No Tide	Total
Number Dead	74	46	120
Number Alive	45	11	56
Total	119	57	176

$\chi^2 = 6.09$, 1df
$p \geq 0.05$

TABLE 2.4-3

CHI-SQUARE CONTINGENCY TABLE COMPARING MORTALITY IN BLACK ABALONE ACCLIMATED TO 16C AND 11C

Acclimation Temperature Produced a Significant Difference in Mortality

	16C-Accl.	11C-Accl.	Total
Number Dead	34	86	120
Number Alive	26	30	56
Total	60	116	176

$\chi^2 = 5.56$, 1df
$p \geq 0.05$

TABLE 2.4-4

CHI-SQUARE CONTINGENCY TABLE COMPARING THERMAL MORTALITY IN MALE VERSUS FEMALE BLACK ABALONE (WITHOUT TIDE)

Mortality of Males is not Significantly Different from that of Females.

	Males	Females	Total
Number Dead	27	16	43
Number Alive	46	41	87
Total	73	57	130

$\chi^2 = 1.1493$, 1 df
$p \geq 0.10$

TABLE 2.4-5

CHI-SQUARE CONTINGENCY TABLE COMPARING THERMAL MORTALITY IN MALE VERSUS FEMALE BLACK ABALONE (WITH AND WITHOUT TIDE)

The Thermal Mortality in Males is Not Significantly Different from Females

	Males	Females	Total
Number Dead	73	47	120
Number Alive	31	25	56
Total	104	72	176

$\chi^2 = 0.47$, 1df
$p \geq 0.10$

Spawning observations during the experiment showed that sperm was nonmotile when released into water above 27.0C, whereas sperm motility appeared unaffected below 27.0C. However, the length of exposure time to the elevated temperature could not be determined and additional information will be gathered on sperm thermal tolerance. Because only one female spawned during the experiment, it was not possible to determine the effects of elevated temperature on released eggs.

The behavioral sequence during heat stress in black abalone was fairly consistent, although all abalone did not exhibit all stages of the response. The first sign of stress was a general loss of body turgor. Secondly, the shell was uplifted from the substrate, usually beginning with a 1-2 cm lift of the anterior end, eventually resulting in elevation of the entire shell 4 cm off the substrate ("gaping"). Gaping was often followed by epipodial spreading. Loss of the ability to hold a surface usually followed epipodial spreading; although, failure to hold did occur at various points. Loss of tentacular responsiveness followed failure to hold a surface. Spawning occurred more frequently at test temperatures than at the control temperatures and occurred at a variety of times during the test period. Abalone rarely moved more than 1-2 cm when heat stressed.

2.4.1.3 DISCUSSION

Ebert (1974) used methods similar to those of the present study to determine the thermal tolerance of red abalone, and his data were used to estimate the 96-hour ET50 of red abalone for comparison to the present results for black abalone. The thermal tolerance of the intertidal species, black abalone, was higher than that of the subtidal species, red abalone. The 96-hour ET50 temperature for continuously submerged abalone was 26.1C (78F) for black abalone acclimated to 11.5C (52.7F), whereas for red abalone acclimated to 10C (50F) it was approximately 24C (75.2F). Acclimation to warmer temperatures resulted in a small but significant increase in thermal tolerance in both species. For black abalone raising the acclimation temperature from 11.5C (52.7F) to 16C (60.8F) increased the ET50 about 1.3 Celsius degrees (2.3 Fahrenheit degrees) whereas for red abalone raising the acclimation temperature from 10 to 20C (50 to 68F) increased the ET50 approximately 1 Celsius degree (1.8 Fahrenheit degrees). A small but significant increase in the ET50 value for black abalone (up to about 0.5 Celsius degrees (0.9 Fahrenheit degrees) resulted from exposure to a tidal cycle, which greatly reduced the "heat dose" during the test period.

The combined effects of thermal history and intertidal elevation significantly affect the thermal tolerance of black abalone. The response to heat stress in black abalone is extremely abrupt, and small changes in temperatures above 25C (77F) are critical. The increase in mortality of black abalone from 0 to 100 percent mortality takes place over only about 1 Celsius degree (1.8 Fahrenheit degrees) in 96 hours.

2.4.2 HIGH TEMPERATURE ACCLIMATION

The results of a 96-hour test to determine the median effective temperature (ET50), i.e., the temperature at which 50 percent of the test population survived, are reported in this section. The individuals used in this experiment were originally collected in November and December 1978 from intertidal areas south of Diablo Cove. They were then placed in a growth experiment which lasted for more than three months (see SECTION 3.5.2 of this report) at the end of which period they were considered to be acclimated to the test temperatures of the growth experiment. Those animals in the 12, 15 and 24 C tanks were subsequently used for these 96-hour tests.

The study objective was to determine the effect of relatively high acclimation temperature on the ET50 value in comparison with responses of animals acclimated to relatively low temperatures. In addition, the time course of mortality was recorded for the 96-hour period. The purpose of this study was to provide information about the possible acclimation of the species to elevated temperatures in Diablo Cove.

2.4.2.1 METHODS

A summary description of the laboratory methods employed in the investigations into heat tolerance for black abalone is presented below. For a more detailed description of the laboratory methods see APPENDIX B (Procedure 3.19) and protocol see APPENDIX C (Experiment 19a).

Moderately large (2-15 cm shell length) black abalone were acclimated to 12, 15 and 24 C. During the experiment, abalone from each of the three acclimation groups were maintained continuously submerged in the laboratory test tanks. Temperature data were recorded hourly by an Autodata Nine computer, transcribed, and summarized as cumulative means and standard deviations at each observation period for each test tank.

Abalone were observed at 12-hour intervals during the 96-hour period at test temperatures ranging from 23.1 to 28.8 C except that the 12 C acclimated animals were not tested at 28.8 C because a previous study (PGandE 1979a) showed that 11 C acclimated animals were 100 percent killed at 27.3 C before 96 hours. Control groups of animals were maintained and observed at 12, 15 and 24 C.

Loss of ability of an abalone to hold to a surface constituted "ecological death" (effective temperature, ET) during the experiment. Abalone which lost the ability to hold were returned to their acclimation temperature to observe any recovery. From eight to 12 abalone were tested in each treatment group. Probit analysis of the mortality data was conducted in accordance with Standard Methods (1975).

2.4.2.2 RESULTS

Over 200 animals with shell lengths ranging from 21.5 to 151.2 mm ($\overline{X}$ = 57.8 mm, SD = 25.3) were used in this experiment. Cumulative means and standard deviations of the temperature data for all test tanks are given in TABLE 2.4-6.

TABLE 2.4-6

BLACK ABALONE HEAT TOLERANCE: CUMULATIVE TEMPERATURE (C) MEANS AND STANDARD DEVIATIONS (IN PARENTHESES) FOR ALL OBSERVATION PERIODS

The data also apply to <u>Haliotis rufescens</u>, <u>Tegula brunnea</u>, <u>Acmaea mitra</u>, <u>Tonicella lineata</u>, <u>Astraea gibberosa</u> and <u>Tegula funebralis</u>, which were run simultaneously with <u>H. cracherodii</u>.

Target Temperature (C)	Observation Period (H)										
	0	4	16	28	40	52	64	76	88	96[a]	162[b]
(Ambient)	12.5	12.8 (0.2)	12.1 (0.6)	12.0 (0.5)	11.8 (0.6)	11.7 (0.6)	11.7 (0.5)	11.7 (0.5)	11.7 (0.5)	11.7 (0.5)	11.1 (0.4)
12	12.0	12.0 (0.0)	12.0 (0.0)	12.0 (0.0)	12.0 (0.0)	12.0 (0.0)	12.0 (0.0)	12.0 (0.0)	12.0 (0.1)	12.0 (0.1)	12.0 (0.0)
15	15.2	15.1 (0.0)	15.1 (0.0)	15.1 (0.0)	15.1 (0.0)	15.1 (0.0)	15.1 (0.0)	15.1 (0.0)	15.1 (0.0)	15.1 (0.0)	14.9 (0.5)
23	23.9	23.3 (0.4)	23.1 (0.2)	23.1 (0.2)	23.1 (0.1)	23.1 (0.1)	23.1 (0.2)	23.1 (0.1)	23.1 (0.1)	23.1 (0.1)	—
24	24.1	24.2 (0.4)	24.0 (0.3)	23.9 (0.3)	23.8 (0.3)	23.8 (0.2)	23.8 (0.3)	23.8 (0.3)	23.9 (0.3)	23.9 (0.3)	24.1 (0.0)
25	24.9	24.9 (0.0)	24.9 (0.0)	24.9 (0.0)	24.9 (0.0)	24.9 (0.0)	24.9 (0.1)	24.9 (0.1)	24.9 (0.1)	24.9 (0.1)	—
26	25.4	25.6 (0.1)	25.8 (0.1)	25.8 (0.1)	25.8 (0.1)	25.8 (0.1)	25.8 (0.1)	25.8 (0.1)	25.8 (0.1)	25.8 (0.1)	—
27	27.0	27.0 (0.0)	27.0 (0.1)	27.0 (0.0)	27.0 (0.0)	27.0 (0.1)	27.0 (0.1)	27.0 (0.0)	27.0 (0.0)	27.0 (0.0)	—
28	28.0	28.1 (0.0)	28.2 (0.1)	28.1 (0.1)	28.1 (0.1)	28.1 (0.1)	28.1 (0.1)	28.1 (0.1)	28.1 (0.1)	28.1 (0.1)	—
29	28.7	28.8 (0.0)	28.8 (0.0)	28.8 (0.0)	28.8 (0.0)	28.8 (0.1)	28.8 (0.0)	28.8 (0.0)	28.8 (0.0)	28.8 (0.0)	—

[a] End of treatment period

[b] End of recovery period

Of the 24 C acclimated animals, only one mortality was observed at 16 hours in the 27.0 C tank. Because no mortalities were observed at 28.1 and 28.8 C, it is assumed that this death is not attributable to temperature, but may possibly have been caused by injury sustained during handling or to natural mortality. A similar case appeared in the 15 C acclimated animals where one death was recorded at 40 hours in the 25.8 C tank even though no deaths were observed in the next higher temperature, 27.0 C. This death is also assumed not to have resulted from temperature stress. Aside from these two deaths, thermal mortality was observed only in the 27.0, 28.1 and 28.8 C tanks. All "dead" animals were replaced into seawater at their acclimation temperature to determine if they would recover. At the end of this recovery period (162 hours) none of the animals had recovered which indicated that the criterion for death during the experiment was valid. The cumulative mortalities for these three tanks at each observation period for the 96-hour test are presented in FIGURE 2.4-4. The 12 C acclimated animals were not tested at 28.8 C because it was determined from a previous study (PGandE 1979a) that these animals would experience 100 percent mortality at lower temperatures.

Probit regression lines were fitted by eye to determine the temperature at 50 percent mortality for the 12 and 15 C acclimated groups at each observation period. Examples of these plots are shown in FIGURE 2.4-5 for the two acclimation groups at 28 and 96 hours. The 96-hour ET50 value was 27.4 C for the 12 C acclimated animals, 28.0 for the 15 C acclimated animals and greater than 29.0 C for the 24 C acclimated animals. Estimations of the temperatures at which 50 percent mortality occurred at each observation period were derived from the probability plots and used to generate curves of time to 50 percent mortality versus temperature shown in FIGURE 2.4-6. The temperature at which the points fall on a vertical line is referred to as the "incipient lethal temperature," that is, the temperature at which just 50 percent of the animals will die when exposed for indefinite periods. As would be expected, the 12 C acclimated animals exhibited lower 96-hour ET50 and incipient lethal temperatures than did the 15 C acclimated animals.

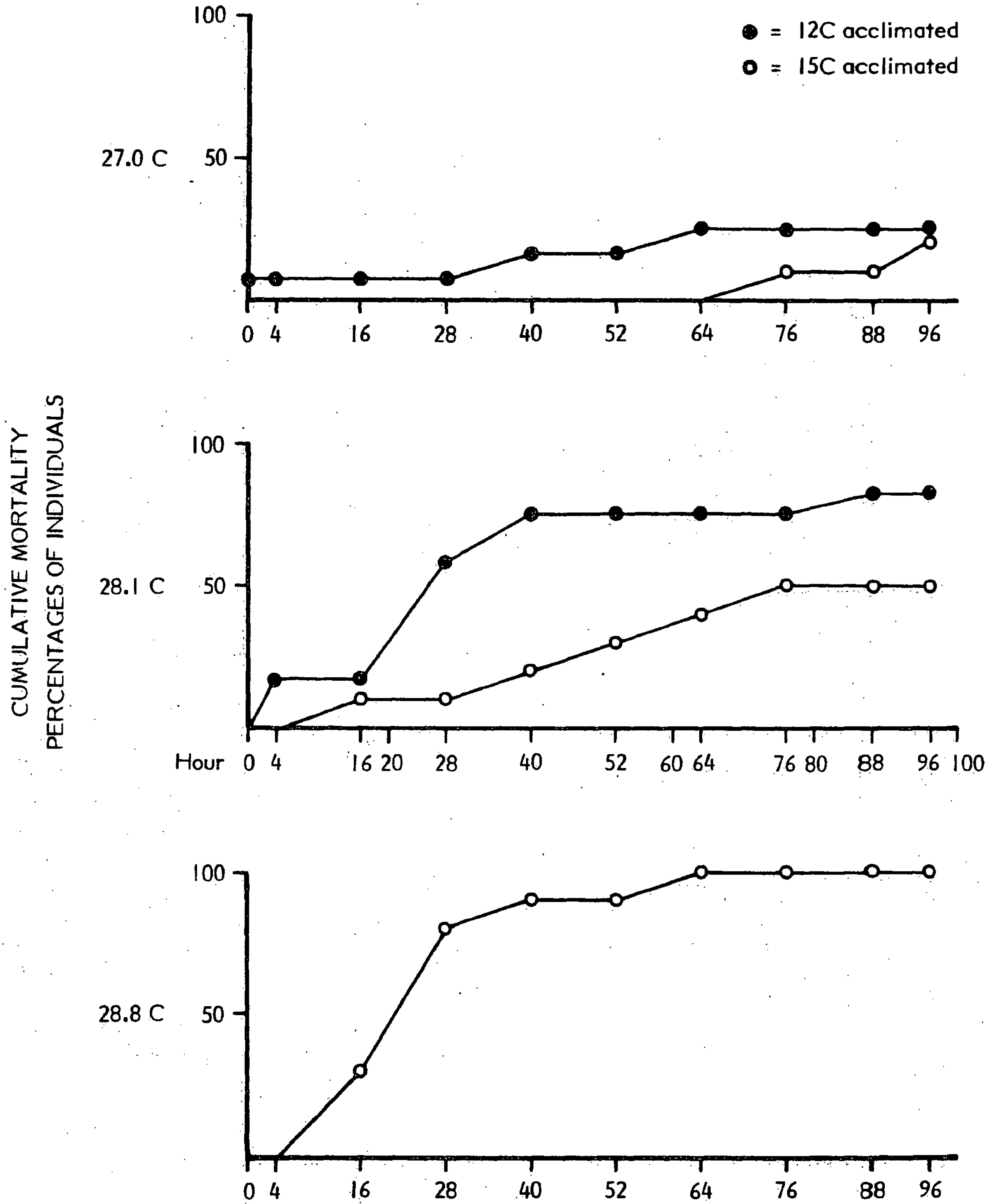

FIGURE 2.4-4

BLACK ABALONE HEAT TOLERANCE:
CUMULATIVE MORTALITY VERSUS TIME

The animals were acclimated to two temperatures and subjected to three test temperatures.

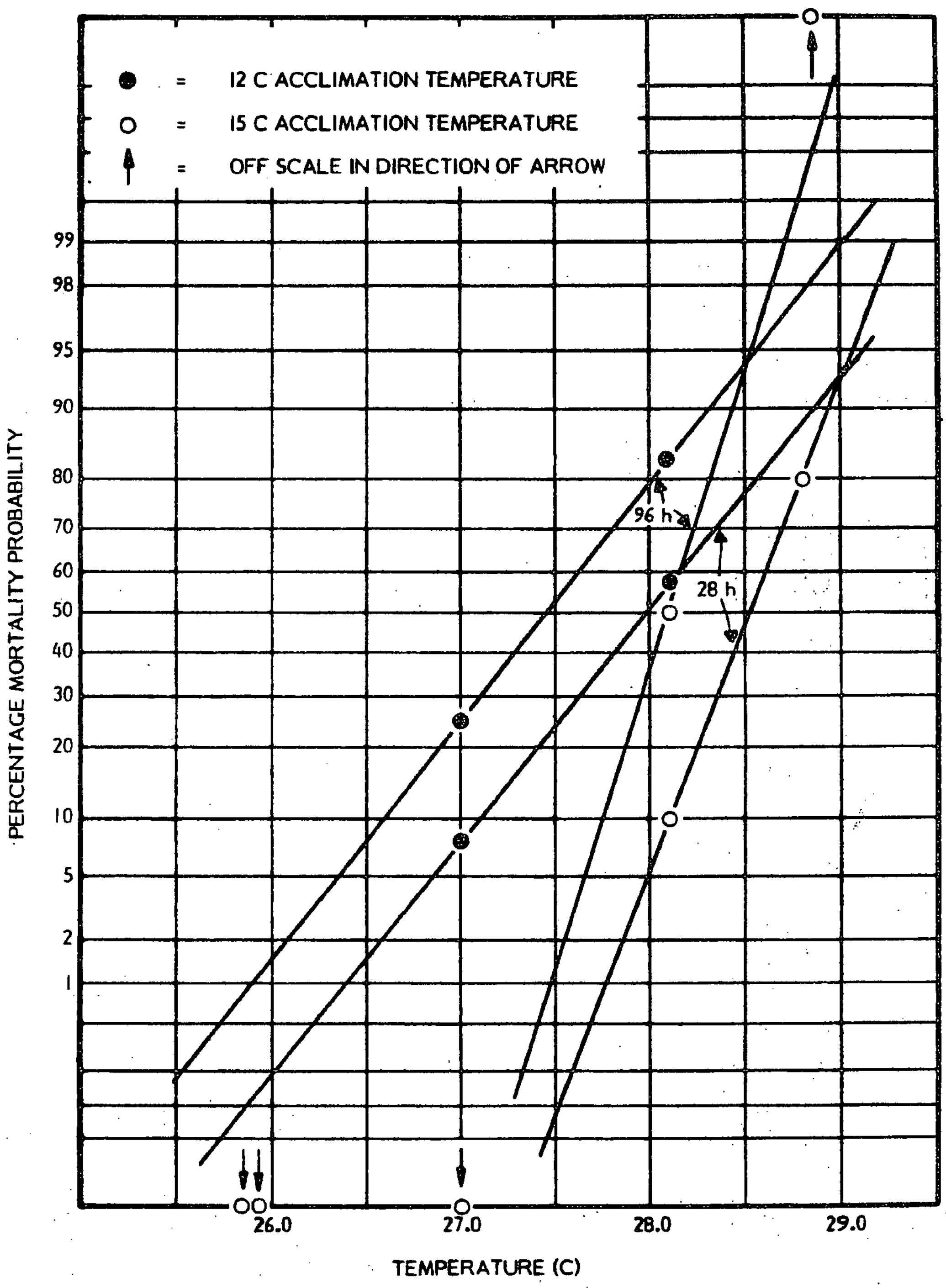

FIGURE 2.4-5

BLACK ABALONE HEAT TOLERANCE:
PERCENTAGE MORTALITY PROBABILITY VERSUS TEMPERATURE AT 28 AND 96 H

The animals were acclimated to two temperatures and exposed to four test temperatures

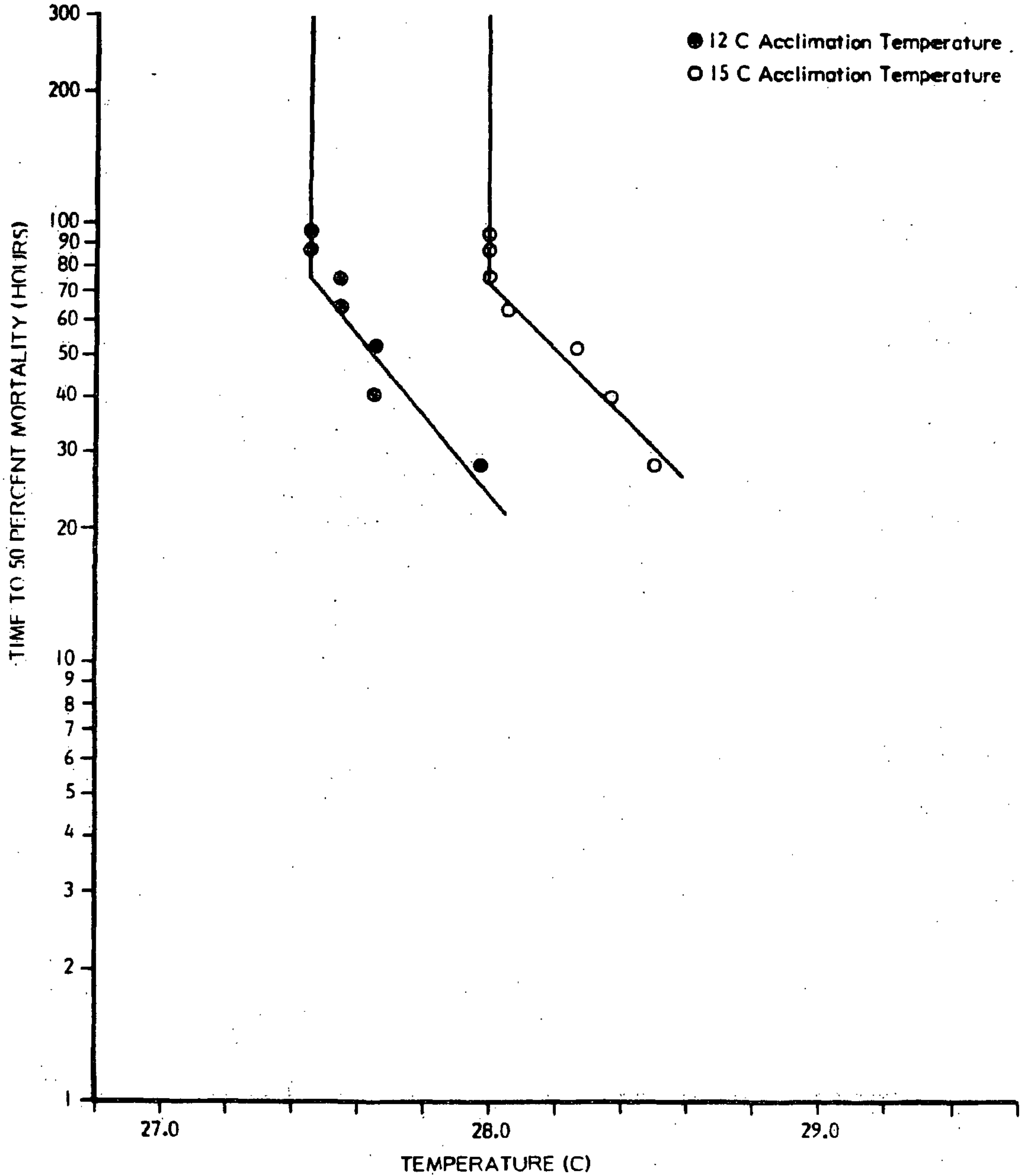

FIGURE 2.4-6

BLACK ABALONE HEAT TOLERANCE:
TIME TO 50 PERCENT MORTALITY

Comparison of these results with those of an earlier study (PGandE 1979a) reveal differences in the ET50 values between groups of similar acclimation temperatures (see TABLE 2.4-7). The 96-hour ET50 values for this experiment are about 1 C higher for 11.5-12 C acclimated animals and about 0.5 C higher for 15-16 C acclimated animals than those obtained in previous experiments.

2.4.2.3 DISCUSSION

The acclimation of black abalone to relatively high temperature (24 C) (75.2 F) has been shown to markedly increase survivability at elevated temperatures and demonstrates that no mortality of 24 C acclimated animals occurs at 29 C (84.2 F) under laboratory conditions. The thermal sensitivity of the animals tested in these experiments appeared to be somewhat lower than that observed in earlier experiments at comparable acclimation temperatures. A possible explanation of this lower sensitivity is that the animals tested in the present experiment had been maintained at constant temperature, continuously submerged and with unlimited food supplies for over three months prior to testing. These conditions may have resulted in a lower sensitivity to elevated temperatures in the ET50 test.

TABLE 2.4-7

BLACK ABALONE HEAT TOLERANCE:

COMPARISON OF 96-H ET50 VALUES
FROM SEVERAL EXPERIMENTS

Experiment Code	Experiment Date	Acclimation Temperature (C)	96-h ET50 (C)
4a	8/17/78	11.5	26.1
19a	4/23/79	12.0	27.4
2a-c	6/2-27/78	16.0	27.4
19a	4/23/79	15.0	28.0
19a	4/23/79	24.0	29.0

2.3 ROCK PRICKLEBACK (XIPHISTER MUCOSUS)

According to Fitch and Lavenberg (1971), the rock prickleback, Xiphister mucosus, inhabits rocky outer coast areas which have a dense algal cover, and it ranges in depth from the intertidal zone to 18.3 m (60 ft). Its geographical distribution extends from Port San Juan, Alaska to the Point Arguello Boat Station, California (Miller and Lea 1972). It reaches a maximum length of 59 cm (25 in.).

The rock prickleback (Xiphister mucosus) is an important species in the ecology of Diablo Cove. It occurs abundantly under rocks in the intertidal region of Diablo Cove (Burge and Schultz 1973). The literature on this species was summarized previously (PGandE 1979b).

Two types of heat tolerance experiments were conducted on rock prickleback juveniles. 96-hour median lethal (LT50) tests and initial thermal maximum (CTM) tests were conducted on 12 and 16 C acclimated animals.

2.3.1 96-HOUR MEDIAN LETHAL TEMPERATURE (96-H LT50)

The study objectives of experiments on the heat tolerance of juvenile rock pricklebacks were the following:

- o To observe the time course of mortality for groups of fish exposed to heated water over a 96-hour period
- o To determine from these observations the temperatures at which 50 percent of the fish die (LT50 values) for standardized 96-hour exposures and for shorter exposures to heated water
- o To measure the effect of acclimation to warm (16 C) and cold (12 C) temperatures on the LT50 values.

The acclimation temperatures were selected to represent average cold season (12 C) and warm season (16 C) water temperatures in the Diablo Canyon area in order to assess the effect of seasonal ambient water temperatures on the species' thermal tolerance. The purpose of this study was to provide information on the effects of the power plant discharge on juvenile rock pricklebacks at different times of the year, when acclimated to different temperatures.

2.3.1.1 METHODS

A summary description of the laboratory methods employed in the investigations of heat tolerance (LT50 determination) of rock pricklebacks was presented in SECTION 2.1.1.1 of this report. For a more detailed description see APPENDIX B Procedure 305. For experiment protocols see APPENDIX C (experiments 5p and 5q). Juvenile rock pricklebacks used in the experiments were collected from Diablo and Field's Coves in November 1979. The fish were taken by hand from the intertidal above the +3 ft level (MLLW). Ambient seawater temperature was approximately 14 C at the time of capture. Upon arrival at the Thermal Effects Laboratory, the pricklebacks were divided haphazardly into two groups of approximately equal numbers. One group was immediately transferred to an acclimation tank at 12 C, the other to an identical tank at 16 C. All animals were fed a daily ration (to excess) of frozen brine shrimp during the acclimation periods. The fish remained in their respective acclimation tanks until the day prior to the commencement of the experiments -an acclimation period of approximately two months.

Probit analysis of mortality data was carried out in accordance with procedures specified in Standard Methods (1976).

2.3.1.2 RESULTS

Juvenile rock pricklebacks acclimated to 12 and 16 C were tested for heat tolerance at a variety of temperatures ranging from 20.1 to 27.7 C. The time courses of mortality for these experiments are shown in TABLES 2.3-1 and 2.3-2. Whereas there was no mortality in the 12 C group at 20 and 22 C, the 16 C group experienced 90 percent and 100 percent mortality, respectively. At a test temperature of 24 C, both acclimation groups succumbed totally, but the 16 C group reached 100 percent mortality 36 hours before the 12 C group. These

TABLE 2.3-1

SUMMARY TEMPERATURE AND THERMAL MORTALITY DATA
FOR 12 C ACCLIMATED ANIMALS:
ROCK PRICKLEBACK HEAT TOLERANCE

Mean Total Length: 138 mm
Mean Wet Weight: 9.2 g

Mean Experiment Temperatures (C):	12.1	20.1	22.0	23.9	25.8	27.6
Standard Deviation:	0.06	0.11	0.22	0.07	0.11	0.36

96 H CUMULATIVE PERCENTAGE MORTALITY

Time (Hours)	Temperature (C)					
	12.1	20.1	22.0	23.9	25.8	27.6
1						
2						50
3						100
4						
5					30	
6					70	
18				10	100	
30				40		
42						
54				60		
66				70		
78				100		
90						
96	0	0	0	100	100	100

TABLE 2.3-2

SUMMARY TEMPERATURE AND THERMAL MORTALITY DATA
FOR 12 C ACCLIMATED ANIMALS:
ROCK PRICKLEBACK HEAT TOLERANCE

Mean Total Length: 137 mm
Mean Wet Weight: 9.2 g

Mean Experiment Temperatures (C):	16.0	20.1	22.0	24.0	25.9	27.7
Standard Deviation:	0.06	0.07	0.16	0.11	0.08	0.36

96 H CUMULATIVE PERCENTAGE MORTALITY

Time (Hours)	Temperature (C)					
	16.0	20.1	22.0	24.0	25.9	27.7
1						
2						100
3						
4						
5						
6						
18					100	
30				50		
42				100		
54		10				
66		40	70			
78		50	100			
90		90				
96	0	90	100	100	100	100

data would indicate that the 16 C acclimated fish are significantly less heat tolerant than the 12 C acclimated fish. The results of 96-hour LT50 experiments on the heat tolerance of black surfperch (see SECTION 2.10.1.1) black-and-yellow/gopher rockfish see SECTION 2.1.1) and blue rockfish (see SECTION 2.2.1) have all shown an increase in heat tolerance with an increase of acclimation temperature from 12 to 16 C. Nevertheless, the rock prickleback's decrease in tolerance with increasing acclimation temperature could be explained if it could be shown that a temperature of 16 C was high enough to be stressful to the animal. In fact, however, the results of a 90-day optimum temperature for growth study (see SECTION 3.4 of this report) indicated that the rock prickleback has its highest growth rate (at constant temperature) between the temperatures of 14 and 18 C; rapid growth would not be considered a reaction to stress induced by temperature. Time course of mortality data from this same growth experiment recorded no deaths at 20 C until after 23 days of exposure.

For each of the observation periods shown in TABLE 2.3-1, cumulative mortalities were plotted on a probability scale versus their respective mean test temperatures. Example plots are shown in FIGURE 2.3-1 of the 6- and 54-hour observations of the 12 C acclimated fish. A line fitted by eye to each of the two plots was used to extrapolate the temperature at which 50 percent mortality would occur for the specified elapsed time period (6 and 54 hours). Those temperatures extrapolated to cause 50 percent mortality at each observation were plotted against time in FIGURE 2.3-2. The temperature at which 50 percent mortality occurs for any length of exposure up to 96 hours can be estimated graphically from this figure. The 96-hour LT50 for juvenile rock pricklebacks acclimated to 12 C was 23.0 C.

2.3.1.3 DISCUSSION

Of the five species of fish tested in this program, only the rock prickleback is a regular inhabitant of the intertidal zone. Knowing of the fluctuations and extremes in temperature found in the intertidal environment, one might initially assume that this species would be more heat tolerant than the other strictly

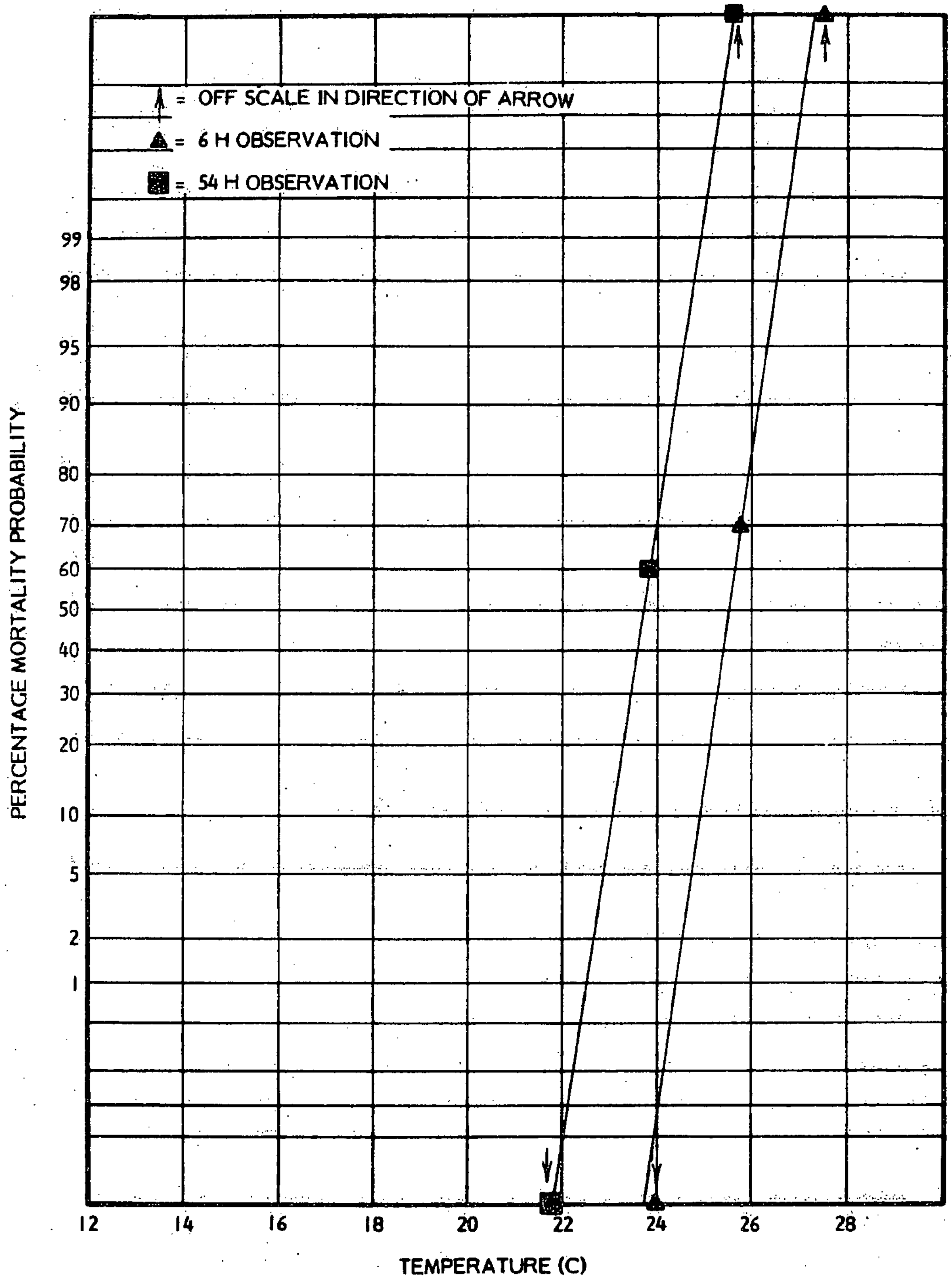

FIGURE 2.3-1

12C ACCLIMATION PERCENTAGE MORTALITY PROBABILITY VERSUS TEMPERATURE AT 6 AND 54 HOUR EXPOSURE: ROCK PRICKLEBACK HEAT TOLERANCE

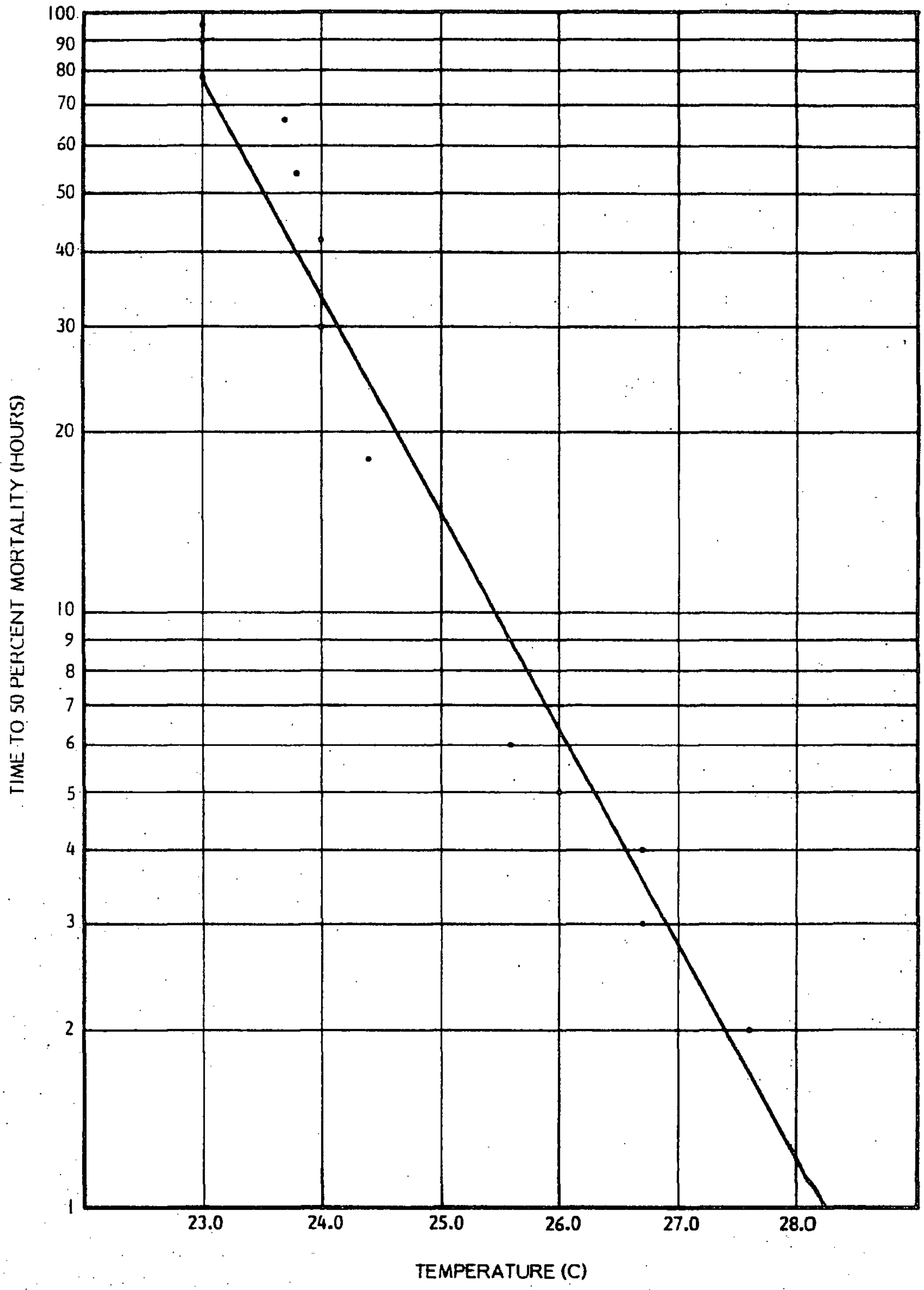

FIGURE 2.3-2

12C ACCLIMATION TIME TO 50 PERCENT MORTALITY: ROCK PRICKLEBACK HEAT TOLERANCE subtidal species. Experimental results do not, however, substantiate this supposition. Of the four fish species that were acclimated to 12 C, only the juvenile blue rockfish had a lower 96-hour LT50 temperature than the rock prickleback. This is most likely reflective of Diablo Canyon's proximity to the rock prickleback's southern range limit at Point Arguello, only 70 km to the south. The ranges of all of the other species tested extend into Baja California, Mexico. It should also be noted that whereas the rock prickleback is an occupant of the intertidal zone, it is not a tidepool dwelling fish, rather it is found beneath rocks and under drift algae in damp,relatively cool areas. Located there, a fish would be insulated from the extremes in temperature which occur at low tide elsewhere in the intertidal region. The results of this experiment indicate that populations of cold season acclimated rock pricklebacks may experience 50 percent mortality when exposed to 23 C (73 F) water for four days.

2.3.2 CRITICAL THERMAL MAXIMUM (CTM)

The objective of the critical thermal maximum experiment was to provide information on the point of mortality of fishes exposed to a rapidly increasing temperature. This standard test provides an index to a species' heat tolerance and can also be used to determine at what point rock prickleback become acclimated to certain temperatures, in preparation for other experiments. The purpose was to provide information about the possible impact of the thermal plume on the species, in particular, the mortality expected when the rock prickleback suddenly encounters relatively high temperatures from which it cannot escape.

2.3.2.1 METHODS

A summary of the methods employed in the critical thermal maximum experiment on rock prickleback was presented in SECTION 2.1.2.1 of this report. The protocol for this experiment is included in APPENDIX C of this report (experiments 8a, b, d, e, f, g, h, and i). A more detailed account of the experimental Procedure 308 used in critical thermal maximum experiments is presented in APPENDIX B (Procedure 308).

The specimens of rock prickleback used in this experiment were collected in November 1979 from Diablo and Field's coves. Those fish used in experiments 8d-i were obtained from the fish growth experiment at its termination (growth experiment temperatures were maintained until CTM testing was initiated).

Each run involved four fish. The runs were made both on fish held at ambient temperatures and on fish that had been acclimated (presumably) to various temperatures.

Because of the versatility of the experiment, it was used to test populations of fish subjected to different holding conditions: (a) ambient (fluctuating) temperatures and (b) acclimated to a constant temperature after being held at that temperature for a long period of time.

A one-way ANOVA test was used to detect significant differences between runs. If significant differences were detected, a posteriori comparisons were performed using Duncan's new multiple range test to determine which of the runs differed.

2.3:2.2 RESULTS

A total of eight CTM experiments, comprising from two to four runs, were conducted on this species. Summary descriptions of the eight experiments follow:

- experiment 8a: two runs, first run 8 January 1980, acclimated to 16C for about 60 days

- experiment 8b: two runs, first run 8 January 1980, acclimated to 12C for about 60 days

- experiment 8d: four runs, first two runs conducted on 19 March 1980, second two runs conducted on 31 March 1980, held at ambient temperatures (13-15.5C prior to the first two runs, but dropping to 10-12.5C immediately thereafter)

- experiment 8e: two runs, conducted on 12 March 1980, acclimated to 12C (via the fish growth experiment)

- experiment 8f: two runs, conducted on 13 March 1980, acclimated to 14C (via the fish growth experiment)

- experiment 8g: two runs, conducted on 18 March 1980, acclimated to 16C (via the fish growth experiment)

- experiment 8h: two runs, conducted on 20 March 1980, acclimated to 18C (via the fish growth experiment)

- experiment 8i: two runs, conducted on 21 March 1980, acclimated to 20C (via the fish growth experiment).

The data from all of these runs are presented in TABLES 2.3-3 through 2.3-7.

2.3.2.3 DISCUSSION

CTM values for ambient held rock prickleback ranged from 29 to 31C (84-88F). Significant differences were detected among the four runs in experiment 8d, which appear to correlate with a change in the ambient holding temperature between the second and third runs (an 11-day period). These results imply that rock prickleback can accommodate changes in temperature within a relatively short period of time.

Significant differences were detected between groups of rock prickleback held and presumably acclimated to constant temperatures for 60-90 days. CTM values increased with increasing holding temperatures up to 16C (61F). However, it appears that the upper limit of CTM value was reached at 16C (61F), in that the value at 18C (64F) was identical to that at 16C (61F) and the value for

TABLE 2.3-3

SUMMARY DATA AND STATISTICAL RESULTS: ROCK PRICKLEBACK
CRITICAL THERMAL MAXIMUM EXPERIMENT 8A

SUMMARY DATA

Run	Mean Length (cm)	Mean Weight (g)	No. Fish	Mean CTM (C)
1	11.0	5.2	5	30.2
2	14.9	13.3	5	30.3
Overall Mean	13.0	9.2	(10)	30.3

STATISTICAL RESULTS

ANOVA: (no significant difference at 0.01 level)

NOTE: The fish had been held at 16C for about 60 days.

TABLE 2.3-4

SUMMARY DATA, STATISTICAL RESULTS AND COMPARISON WITH 16C HELD ANIMALS: ROCK PRICKLEBACK CRITICAL THERMAL MAXIMUM EXPERIMENT 8B

SUMMARY DATA

Run	Mean Length (cm)	Mean Weight (g)	No. Fish	Mean CTM (C)
1	12.1	6.7	5	29.6
2	10.4	4.5	5	29.6
Overall Mean	11.2	5.6	(10)	29.6

STATISTICAL RESULTS

ANOVA: (no significant difference at 0.01 level)

COMPARISON WITH 16C-HELD ANIMALS

ANOVA: $F_{(3,16)} = 12.162$ (significant)

Duncan's Test (bars connect runs not significantly different)

CTM (C)	29.6	29.6	30.2	30.3
	12C		16C	

NOTE: The fish had been held at 12C for about 60 days.

TABLE 2.3-5

SUMMARY DATA AND STATISTICAL RESULTS: ROCK PRICKLEBACK CRITICAL THERMAL MAXIMUM EXPERIMENT 8D

SUMMARY DATA[a]

Run	Mean Length (cm)	Mean Weight (g)	No. Fish	Mean CTM (C)	Day
1	95	3.3	5	30.5	1
2	100	3.2	5	30.6	1
3	104	4.1	5	30.1	12
4	111	5.0	5	29.9	12

STATISTICAL RESULTS

ANOVA: $F_{(3,16)} = 40.828$ (significant at 0.01 level)

Duncan's Test (bars connect runs not significantly different)

CTM (C)	29.9	30.1	30.5	30.6
Run	4	3	1	2

[a] In the 19 days prior to runs 1 and 2, mean temperature was 13.5C (maximum 15.4, minimum 12.8C). For the period between runs 1/2 and 3/4, mean temperature was 11.1C (maximum 12.6, minimum 10.3C).

NOTE: The fish were held at ambient temperatures prior to all runs.

TABLE 2.3-6

SUMMARY DATA: ROCK PRICKLEBACK
CRITICAL THERMAL MAXIMUM EXPERIMENTS 8E, 8F, 8G, 8H, AND 8I
ON FISH HELD IN THE 90-DAY GROWTH EXPERIMENT

Experiment	Run	Mean Length (cm)	Mean Weight (g)	No. Fish	Mean CTM	Holding Temperature (C)
8e	1	93	2.7	5	30.1	12
	2	84	2.1	5	30.3	12
8f	1	89	2.6	5	30.3	14
	2	99	3.6	5	30.5	14
8g	1	84	2.3	5	30.8	16
	2	77	1.7	5	30.9	16
8h	1	96	3.8	5	30.8	18
	2	86	2.7	5	30.8	18
8i	1	82	2.3	5	30.7	20
	2	79	1.9	5	30.4	20

TABLE 2.3-7

STATISTICAL RESULTS: ROCK PRICKLEBACK
CRITICAL THERMAL MAXIMUM EXPERIMENTS 8E, 8F, 8G, 8H, AND 8I
ON FISH HELD IN THE 90-DAY GROWTH EXPERIMENT

Test	Experiment	Accl. Temp. (C)	Result
ANOVA:	8e	12	$F_{(1,8)} = 5.565$ (significant at 0.01)
	8f	14	$F_{(1,8)} = 5.400$ (significant at 0.01)
	8g	16	$F_{(1,8)} = 2.000$ (not significant at 0.01)
	8h	18	$F_{(1,8)} = 0.100$ (not significant at 0.01)
	8i	20	$F_{(1,8)} = 0.2159$ (not significant at 0.01)

Duncan's Test (bars connect runs not significantly different) [a]

Acclimation Temperature (C)	12	14	16	18
CTM (C)	30.2	30.4	30.8	30.8

[a] Experiment 8i was not analyzed with Duncan's Test because of the large degree of variance in the data.

20C (68F) held rock prickleback was lower and exhibited a higher variance. These results are particularly interesting in light of the fact that significant mortality occurred in the rock prickleback population in the 20C (68F) tank of the fish growth experiment (21 of 40 fish died during the 90-day experimental period), and the CTM fish tested were taken from the 21 surviving fish. The CTM values ranged from 30 to 31C (86-88F) over the holding temperatures of 12 to 20C (54-68F); therefore, even the significant differences detected are relatively small in magnitude.

2.5 RED ABALONE (HALIOTIS RUFESCENS) 96-HOUR MEDIAN EFFECTIVE TEMPERATURE (96-H ET50)

The red abalone (Haliotis refescens) is an important species in the ecology of Diablo Cove. A review of the literature on the red abalone was presented in PGandE (1979b). The results of 96-hour tests to determine the median effective temperature (ET50), i.e., the temperature at which 50 percent of the test population survived, are reported on in this section.

The study objective was to determine the effect of relatively high acclimation temperature on the ET50 value in comparison with responses of animals acclimated to relatively low temperatures. In addition, the time course of mortality was recorded for the 96-hour period. The purpose of this study was to provide information about the possible acclimation of the species to elevated temperatures in Diablo Cove.

2.5.1 METHODS

A summary description for the laboratory methods employed in the investigations into heat tolerance for red abalone is presented below. For a more detailed description of the laboratory methods see APPENDIX B (Procedure 319) and protocol see APPENDIX C (Experiment 19a).

The individuals used in this experiment were originally obtained in December 1977 from California Marine Associates as approximately 2 cm long hatchery reared juveniles. The animals were maintained in the PGandE Biolab on the Diablo Canyon site at ambient temperatures and were fed Macrocystis to excess. They were then placed in a growth experiment which lasted for more than three months (see SECTION 3.5.2 of this report) at the end of which period they were considered to be acclimated to the test temperatures of the growth experiment.

Those animals in the 12, 15 and 24 C tanks were subsequently used for this 96-hour test. During the experiment, abalone from each of the three acclimation groups were maintained continuously submerged in the laboratory test tanks.

Temperature data were recorded hourly by an Autodata Nine computer, transcribed, and summarized as cumulative means and standard deviations at each observation period for each test tank.

Abalone were observed at 12-hour intervals during the 96-hour period at test temperatures ranging from 23.1 to 28.8 C except that the 12 C acclimated animals were not tested at 28.8 C and the 24 C acclimated animals were not tested at 23.1 or 24.9 C because of lack of sufficient number of individuals. Control groups of animals were maintained and observed at 12, 15 and 24 C.

Loss of ability of an abalone to hold to a surface constituted "ecological death" (effective temperature, ET) during the experiment. Abalone which lost the ability to hold were returned to their acclimation temperature to observe any recovery. From five to seven abalone were tested in each treatment group. Probit analysis of the mortality data was conducted in accordance with Standard Methods (1975).

2.5.2 RESULTS

Over 200 animals with shell lengths ranging from 49.0 to 93.2 mm (X = 63.8 mm, SD = 10.6) were used in this experiment. Cumulative means and standard deviations of the temperature data for all test ranks are given in TABLE 2.4-6 (see SECTION 2.4.2).

Mortality data for the experiment are presented in FIGURE 2.5-1. In the 12 C acclimated animals, 33 percent mortality was recorded at the end of the 96-hour period in the lowest treatment temperature, 23.1 C, whereas 100 percent mortality was already evident at the zero observation (i.e., after the temperature adjustment period) in the 27 and 28.1 C treatments. In the 15 C acclimated animals, 20 percent mortality was recorded in the 23.1 C treatment at 96 hours and 100 percent mortality was recorded at the zero observation period in the 28.1 and 28.8 C treatments. In the 24 C acclimated animals, no mortality was recorded in the lowest temperature treatment tested (23.9 C) but 17 percent mortality resulted by 96 hours in the 25.8 C treatment and 100 percent mortality

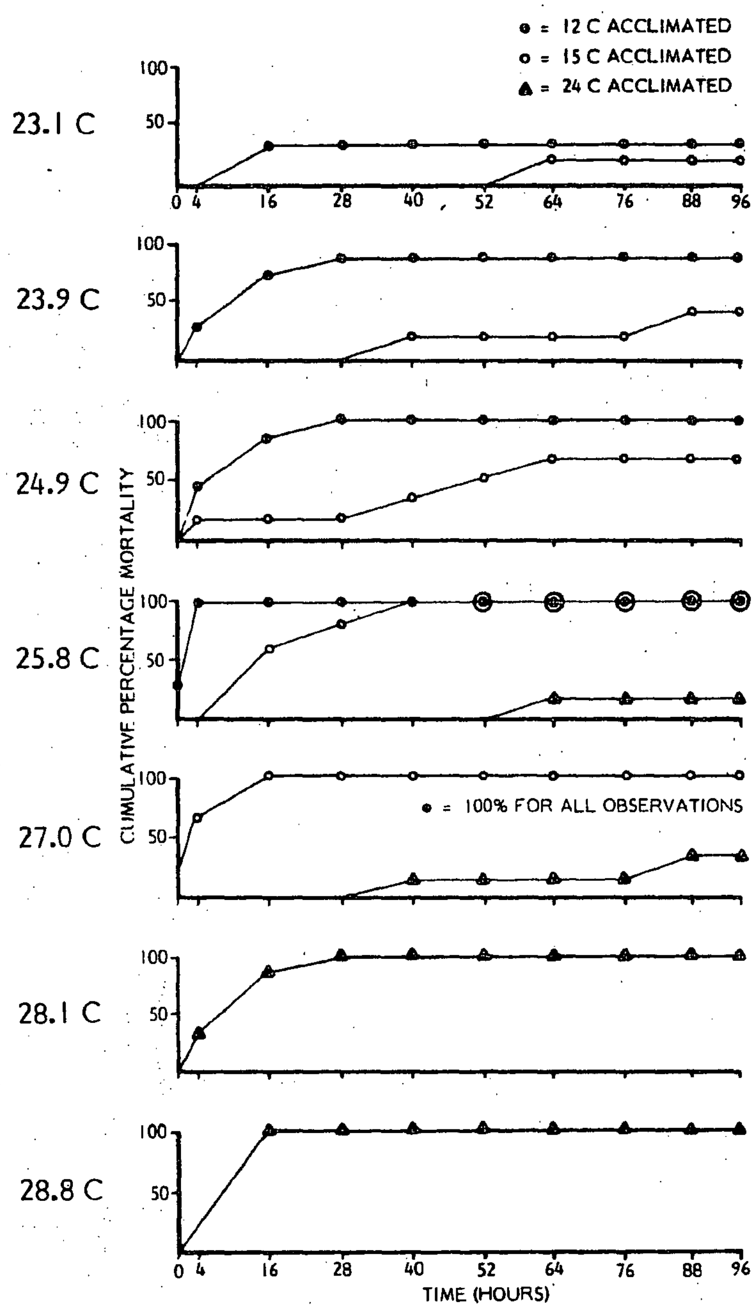

FIGURE 2.5-1

RED ABALONE HEAT TOLERANCE:
CUMULATIVE MORTALITY VERSUS TIME

The animals were acclimated to three temperatures. Mortalities of 100 percent were recorded at all observation periods for the 12 C acclimated animals at 27.0 and 28.1 C and for the 15 C acclimated animals at 28.1 and 28.8 C.

was reached by 96 hours in the 28.1 C treatment. All animals observed to be dead in the treatment tanks were immediately placed into their respective acclimation temperature to determine whether recovery would occur. None of these animals had recovered by 162 hours. All test animals still alive at the end of the 96-hour test period were returned to their acclimation temperature for a further 96 hours to determine "latent mortality." Only two latent mortalities were recorded: one 15 C acclimated animal from the 15 C control tank (which is assumed not to be attributable to the effect of temperature) and one 24 C acclimated animal from the 25.8 C treatment. The latter mortality most likely can be attributed to the effect of temperature but because it did not occur within the the 96-hour period, it will not be included in the following analysis.

Probit regression lines were fitted by eye to determine the temperature at 50 percent mortality at each observation period for each of the acclimation groups. An example of these plots is given in FIGURE 2.5-2 for the 40-hour observation. The 96-hour ET50 value was estimated to be 23.4 C for the 12 C acclimated animals, 24.3 C for the 15 C acclimated animals and 27.1 C for the 24 C acclimated animals. Estimations of the temperatures at which 50 percent mortality occurred at each observation period were derived from the probability plots and used to generate curves of time to 50 percent mortality versus temperature shown in FIGURE 2.5-3. The temperature at which the points fall on a vertical line is referred to as the "incipient lethal temperature," that is, the temperature at which just 50 percent of the animals at a certain acclimation temperature will die when exposed for indefinite periods. Incipient lethal temperatures (which are here equivalent to the 96-hour ET50 values) were defined for all three acclimation groups. As would be expected, the 96-hour ET50 and incipient lethal temperature increases as the acclimation temperature increases.

In the only paper dealing with heat tolerance of red abalone, Ebert (1974) presented observations on mortality of animals acclimated to 14 C. He did not estimate a 96-hour ET50, but in 120-hour exposures, observed that survival was 80 percent at 22 C and only 20 percent at 23 C. From these data, an estimate of

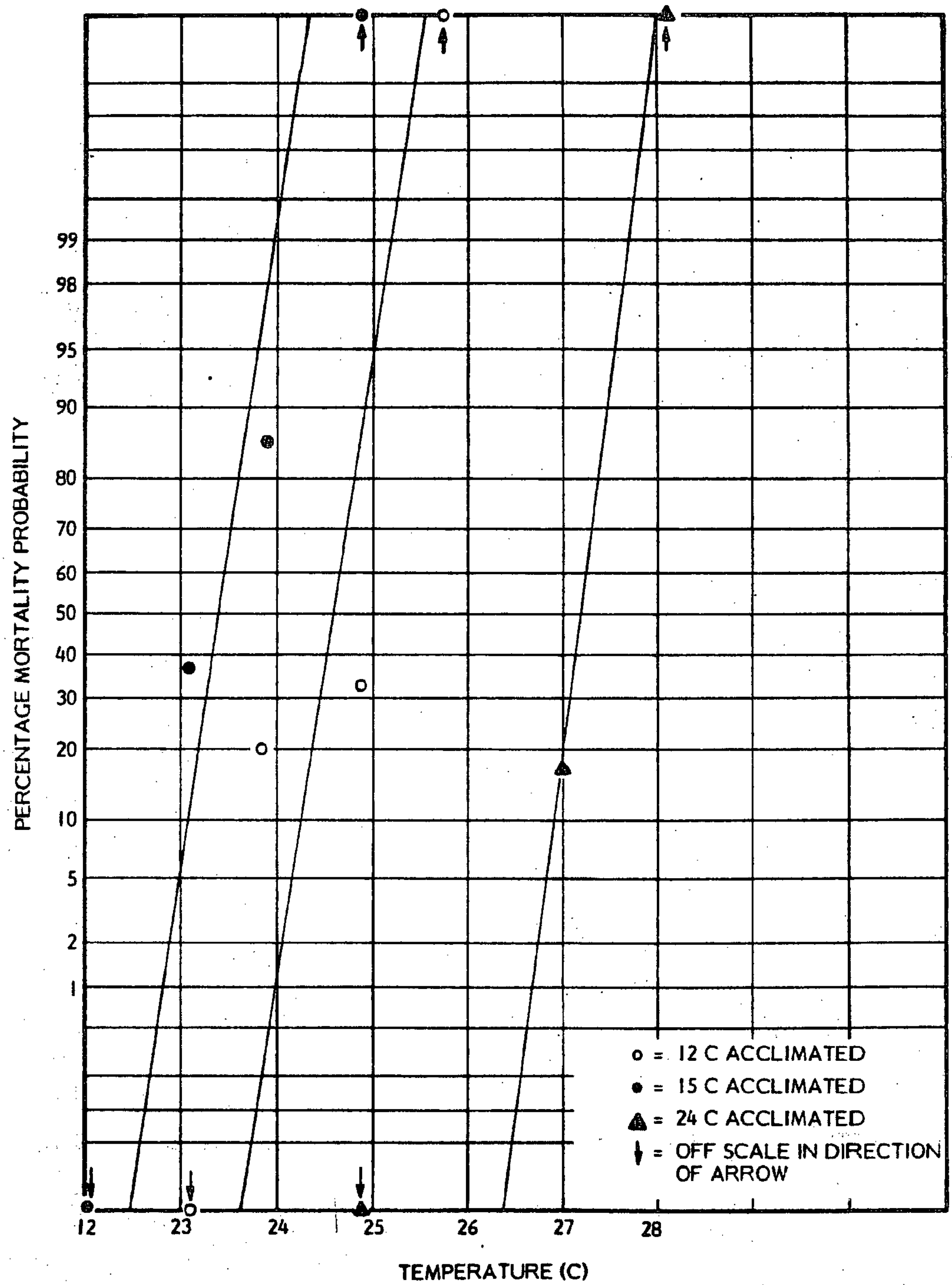

FIGURE 2.5-2

RED ABALONE HEAT TOLERANCE:
PERCENTAGE MORTALITY PROBABILITY VERSUS TEMPERATURE AT 40 H

The animals were acclimated to three temperatures.

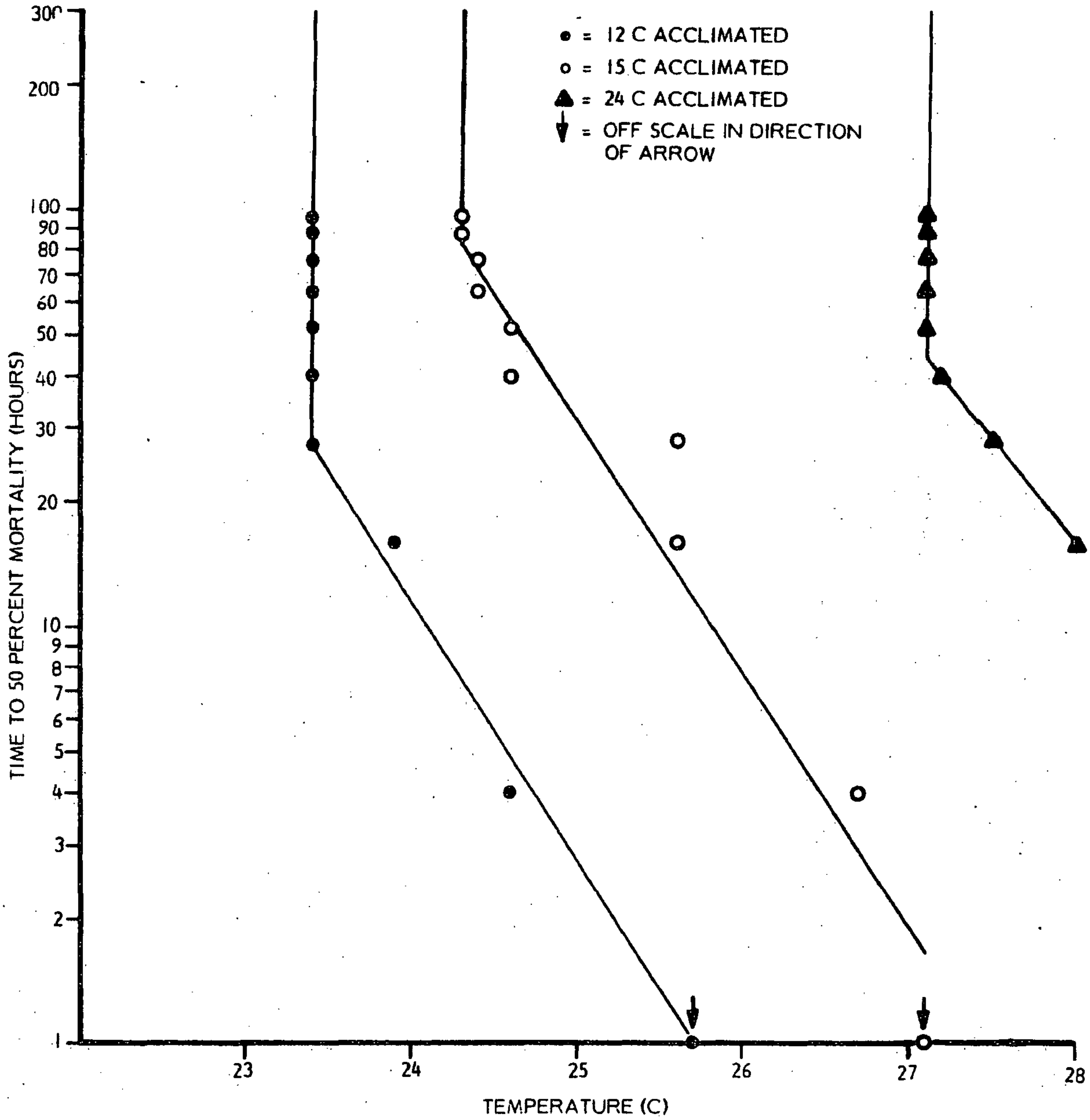

FIGURE 2.5-3

RED ABALONE HEAT TOLERANCE:
TIME TO 50 PERCENT MORTALITY

the 96-hour ET50 value would fall at about 23-24 C, which is in good agreement with the results presented above.

2.5.3 DISCUSSION

The acclimation of red abalone to relatively high temperature (24 C) (75.2F) has been shown to markedly increase survivability at elevated temperatures. In animals acclimated to 24 C (75.2 F), less than 50 pecent of the population would be expected to die at temperatures below 27.1 C (80.8 F). However, natural populations may respond more nearly like the 12 or 15 C (53.6 or 59.0 F) acclimated animals of this experiment, in which case, less than 50 percent of the population would be expected to die at 23.4 and 24.3 C (74.1 and 75.7 F), respectively. These results demonstrate that red abalone are more sensitive to increased temperature than are black abalone (see SECTION 2.4.2). This difference appears logical in that natural populations of black abalone are primarily intertidal in distribution and are thus subjected to greater extremes of temperature than are red abalone populations which are primarily subtidal in distribution.

2.6 ROCK CRAB (CANCER ANTENNARIUS) 96-HOUR MEDIAN EFFECTIVE TEMPERATURE (96-H ET50)

A review of the literature on the rock crab, Cancer antennarius, was presented in PGandE 1979b. This section reports the results of a 96-hour ET50 test to determine the median effective temperature (ET50). The ET50 is the temperature at which 50 percent of the test population survives for the duration of the test. The organisms were collected by trapping from subtidal areas in the vicinity of Diablo Canyon. They were held in the laboratory at ambient temperature prior to the test. The objective of this experiment was to determine the 96-hour ET50 temperature for this species as an index to its thermal tolerance. The time course of mortality throughout the experiment was recorded. There are no published accounts of the effects of elevated temperatures on this species.

2.6.1 METHODS

A summary of the methods employed in the heat tolerance experiment on this species is presented below. A more detailed account of the experiment procedure utilized in 96-hour ET50 experiments is presented in APPENDIX B (Procedure 319). The protocol for this experiment is included in APPENDIX C of this report (experiment 19C).

Specimens tested included both juveniles and adults that were collected in the field by trapping and were acclimated to laboratory conditions at ambient temperatures prior to testing. During the experiment crabs were kept completely submerged in test tanks which were subdivided to accommodate several species per test. Temperature was controlled and recorded by an Autodata Nine computer. Temperature data were transcribed and summarized as cumulative means and standard deviations for each observation period.

Observations were made after six hours, and subsequently every 12 hours for the 96-hour test period. Experimental target temperatures were 19, 20.5, 22, 23.5, 25, 26.5 and 28 C. An ambient control (mean of 12.5 C) was also run. Test populations consisted of ten animals per treatment. The criterion for death was loss of the ability to walk when gently prodded. Animals that were presumed dead were returned to an ambient temperature tank to assess possible recovery. Mortality data were analyzed with probit analysis in accordance with Standard Methods (1976).

2.6.2 RESULTS

Cumulative means and standard deviations of temperature data for each observation period are presentd in TABLE 2.6-1.

Mortality occurred at 22, 26.5, and 28 C (FIGURE 2.6-1). At 22 C, only one mortality occurred throughout the course of the experiment. At 26.5 C 90 percent mortality was reached after 30 hours, and at 28 C 100 percent mortality was observed after six hours. None of the presumed dead animals recovered after being placed in the ambient temperature recovery tank.

Probit regression lines were fitted by eye to the mortality data to determine the temperature which would cause 50 percent mortality at each observation period (FIGURE 2.6-2). The 96-hour ET50 value was determined to be 25.4 C. ET50 estimators for each observation period were used to generate a curve of time to 50 percent mortality versus temperature, which is presented in FIGURE 2.6-3. The "incipient lethal" temperature, indicated by the vertical alignment of points to the left of the curve, was defined by the experiment to be 25.4 C, which is equivalent to the 96-hour ET50 value.

2.6.3 DISCUSSION

Based on these results, seawater temperatures in excess of 25.4 C (77.7 F) for four days would be expected to cause at least 50 percent mortality in a population of rock crabs. Little or no thermal mortality would be expected for exposures to temperatures below 25 C (77 F) for the same period of time.

TABLE 2.6-1

CUMULATIVE TEMPERATURE MEANS AND STANDARD DEVIATIONS (IN PARENTHESES) FOR ALL OBSERVATION PERIODS: ROCK CRAB HEAT TOLERANCE

These data also apply to 96-hour ET50 tests on _Pugettia producta_, _P. richii_, _Pachygrapsus crassipes_, and _Hemigrapsus nudus_.

Target Temperature (C)	Observation Period (Hours)									
	0	6	18	30	42	54	66	78	90	96
Ambient	13.1 (0.0)	13.3 (0.1)	12.7 (0.5)	12.6 (0.5)	12.4 (0.6)	12.3 (0.6)	12.3 (0.5)	12.5 (0.7)	12.6 (0.7)	12.6 (0.7)
19	18.7 (0.0)	19.0 (0.1)	18.9 (0.1)	19.0 (0.1)	19.0 (0.1)	19.0 (0.1)	19.0 (0.1)	19.0 (0.1)	19.0 (0.1)	19.0 (0.1)
20.5	20.6 (0.0)	20.6 (0.0)	20.6 (0.0)	20.6 (0.0)	20.6 (0.0)	20.6 (0.0)	20.6 (0.0)	20.6 (0.0)	20.6 (0.0)	20.6 (0.0)
22	21.8 (0.0)	22.1 (0.1)	22.0 (0.1)	22.0 (0.1)	22.0 (0.1)	22.0 (0.1)	22.0 (0.1)	22.0 (0.1)	22.0 (0.1)	22.1 (0.1)
23.5	23.3 (0.0)	23.5 (0.1)	23.5 (0.1)	23.5 (0.1)	23.5 (0.1)	23.5 (0.1)	23.5 (0.1)	23.5 (0.1)	23.5 (0.1)	23.5 (0.1)
25	24.8 (0.0)	24.9 (0.1)	24.9 (0.1)	24.9 (0.1)	24.9 (0.1)	24.9 (0.1)	24.9 (0.1)	24.9 (0.1)	24.9 (0.0)	24.9 (0.0)
26.5[a]	26.4 (0.0)	26.5 (0.0)	26.5 (0.0)	26.5 (0.0)	26.5 (0.0)	26.5 (0.0)	26.5 (0.0)	26.5 (0.0)	26.5 (0.0)	26.5 (0.0)
28	28.0 (0.0)	28.0 (0.0)	27.9 (0.0)	28.0 (0.0)	28.0 (0.0)	28.0 (0.1)	28.0 (0.1)	28.0 (0.1)	28.0 (0.1)	28.0 (0.1)
	0	12	24	36	48	60	66	96		
26.5[b]	26.5 (0.0)	26.4 (0.0)	26.4 (0.0)	26.4 (0.0)	26.5 (0.1)	26.5 (0.0)	26.5 (0.0)	26.6 (0.1)		

[a] Run 1
[b] Run 2

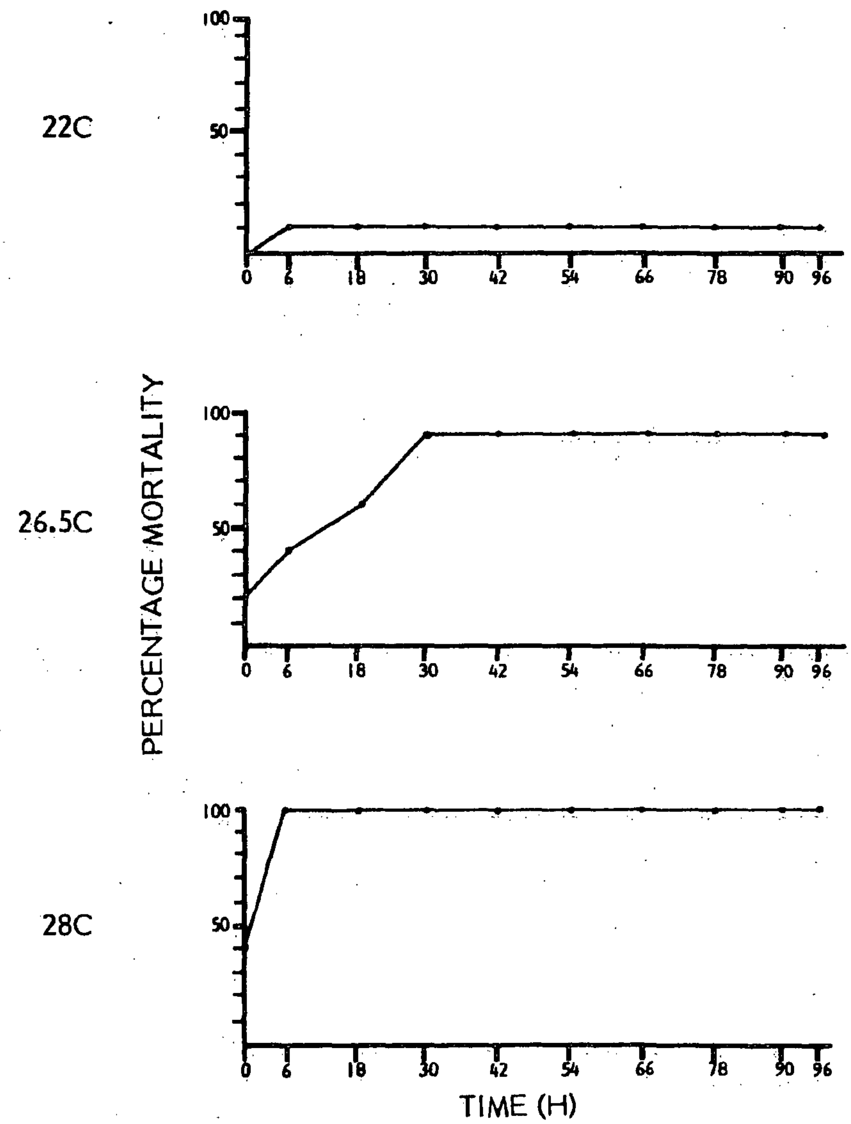

FIGURE 2.6-1

CUMULATIVE MORTALITY VERSUS TIME:
ROCK CRAB HEAT TOLERANCE

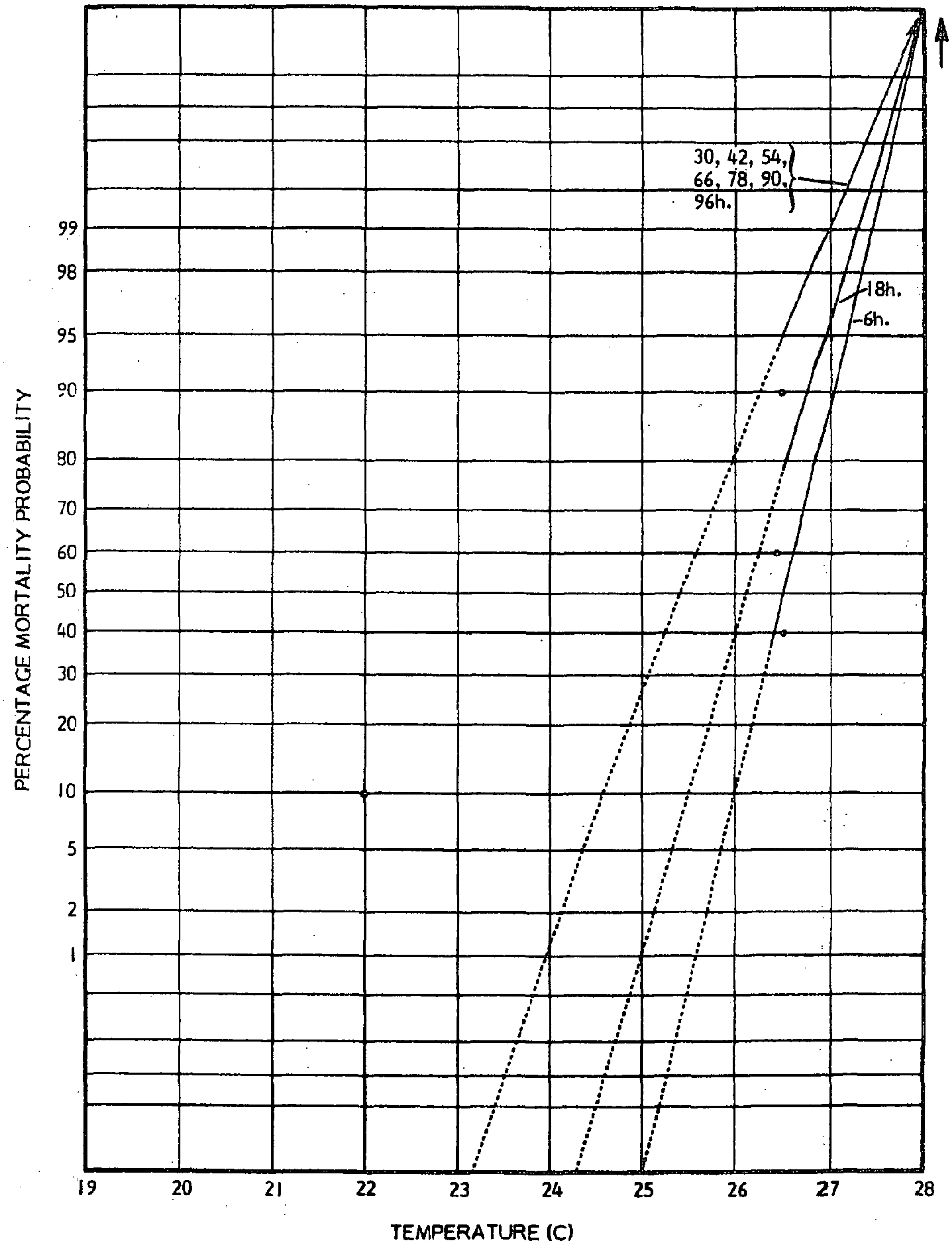

FIGURE 2.6-2

PERCENTAGE MORTALITY PROBABILITY VERSUS TEMPERATURE: ROCKCRAB HEAT TOLERANCE

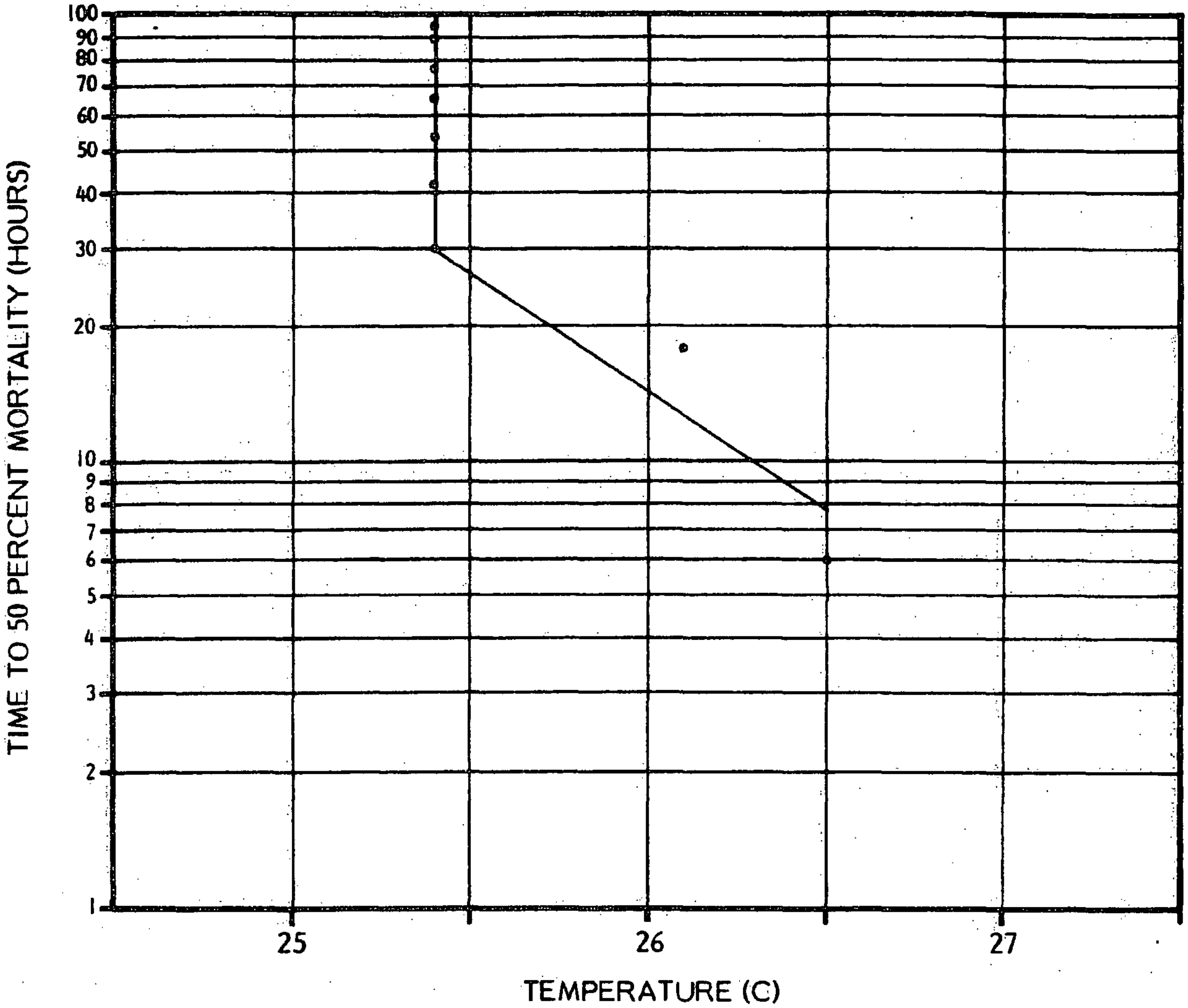

FIGURE 2.6-3

TIME TO 50 PERCENT MORTALITY VERSUS TEMPERATURE:
ROCK CRAB HEAT TOLERANCE

2.7 BULL KELP (NEREOCYSTIS LUETKEANA) SPOROPHYTE 44-DAY MORTALITY

Information concerning thermal tolerance of bull kelp (Nereocystis luetkeana) sporophytes was gathered from mortality data collected during the course of the bull kelp sporophyte growth experiment. Observations of thallus condition (i.e., bleaching and turgor) were not recorded, nor were vital staining observations made. Conclusions are based solely on observed mortality.

2.7.1. METHODS

A summary description of the laboratory methods employed in the bull kelp sporophyte growth experiment is presented in SECTION 3.7.3. For a more detailed description of the laboratory methods see APPENDIX B (Procedure 311) and protocol see APPENDIX C (Experiment 11b).

The following criteria were employed in designation of plant mortality: disintegration of entire blade, pneumatocyst, and distal portions of stipe; mechanically severed stipe; and mechanically broken pneumatocyst.

2.7.2 RESULTS

The mean temperatures of the seawater in the tanks for the 44-day period were: 12.8 (ambient), 10.2, 11.8, 15.9, 18.0, 18.8 and 20.0 C. The mortality data are presented in FIGURE 2.7-1. No mortalities were observed in either the ambient or 10.2 C treatments. At 11.8 C four deaths unrelated to thermal stress occurred between 28 and 44. In the 15.9 C treatment, one mortality was observed at day 40, and two more on day 44.

Significantly higher mortality rates were observed in the 18.0, 18.8 and 20.0 C treatments. At 18.0 C, ten plants died between days 28 and 44, at 18.8 C, 20 mortalities had occurred by day 28, and at 20.0 C, 23 had occurred by day 16.

	NUMBER OF DAYS											
TEMPERATURE (C)	00	04	08	12	16	20	24	28	32	36	40	44	CUMULATIVE MORTALITY
12.8 (AMBIENT)													0
10.2													0
11.8								1	2			1	4
15.9											1	2	3
18.0								2	2	4		2	10
18.8					2	3		15					20
20.0			1	8	14								23

FIGURE 2.7-1

NUMBER OF DEAD PLANTS OBSERVED IN EACH TREATMENT
AT EACH SAMPLING PERIOD:
BULL KELP SPOROPHYTE HEAT TOLERANCE

Percentage mortality values are presented graphically in FIGURE 2.7-2. Values were calculated from the total number of mortalities divided by number of plants theoretically existing in a sample population at the specified time (i.e., on day 12, sample size equaled 30 plants, on day 28, 20 plants, and day 44, 10 plants). As can be seen, 100 percent mortality had occurred at 18.8 and 20.0 C by day 28; 100 percent mortality had occurred in the 18.0 C treatment by day 40 and 30 percent mortality had occurred in both 11.8 and 15.9 C treatments by day 44.

2.7.3 DISCUSSION

In the laboratory, juvenile sporophytes of bull kelp cannot endure prolonged exposure to water temperatures of 18.0 C (64.4 F) and above. At 44 days, over 25 pecent mortality resulted in the 11.8 and 15.9 C (53.2 and 60.6 F) treatments. These mortalities were caused by mechanical damage to either stipe or pneumatocyst. At 11.8 C (53.2 F), sporophytes rapidly outgrew the size at which they were conveniently handled in the raceways (the highest growth rates were observed at 11.8 C (53.2 F): refer to SECTION 3.7.3) causing tissue abrasion, wounding, and eventual death. Therefore, at least some of the mortality at 11.8 and 15.9 C (53.2 and 60.6 F) can be attributed to handling effects. Similar phenomena occurred at 15.9 C (60.6 F), though the plants in this treatment were not so large as those at 11.8 C (53.2 F). Visual examination indicated a reduced capacity for wound-healing in plants at 15.9 C (60.6 F).

No mortalities occurred in either the 10.2 C (50.4 F) or ambient temperatue treatments indicating that mortalities observed at higher temperatures were not a result of negative reactions to handling or to laboratory conditions.

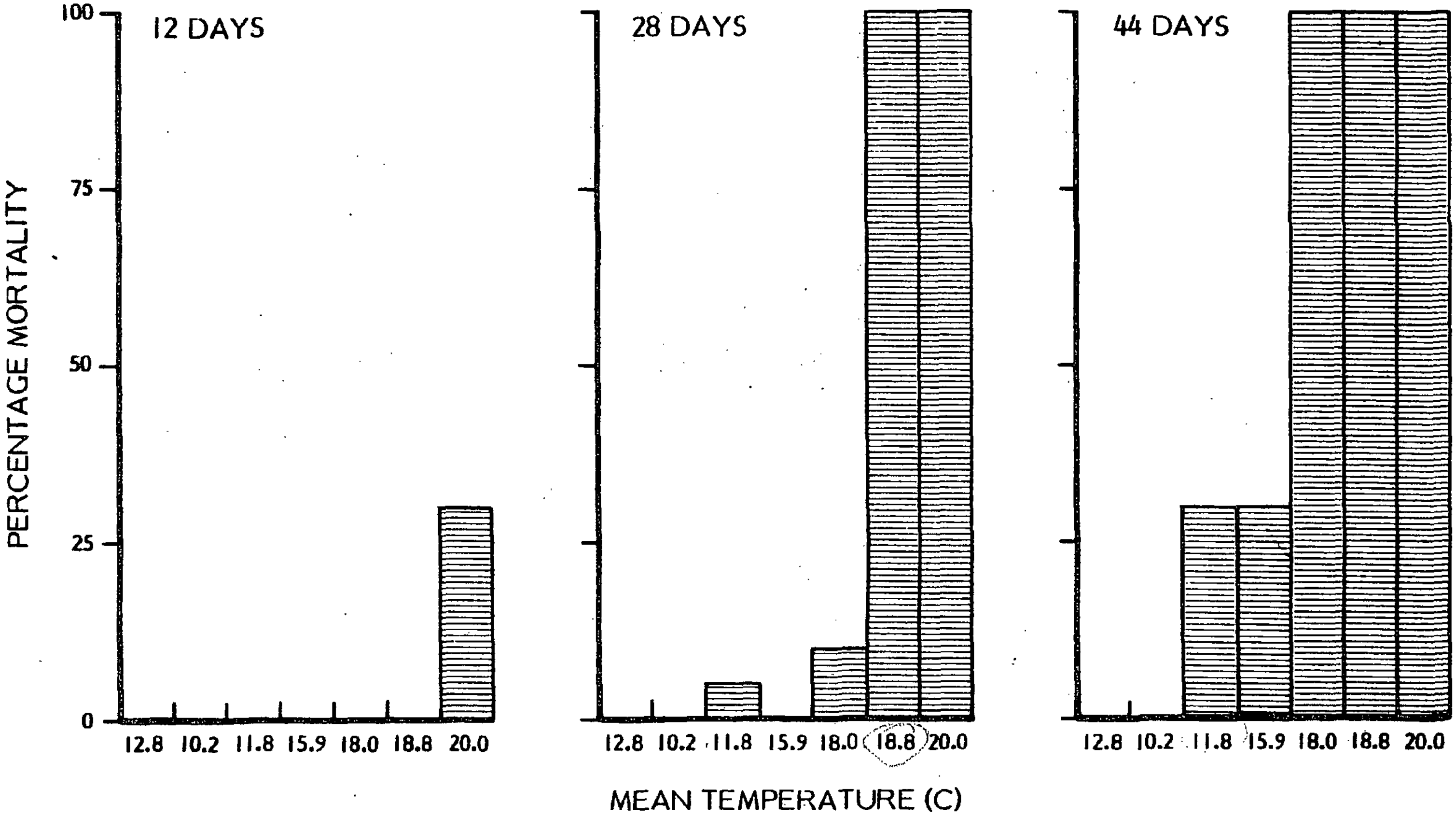

FIGURE 2.7-2

PERCENTAGE MORTALITY VERSUS TEMPERATURE
AT THREE SAMPLING PERIODS:
BULL KELP SPOROPHYTE HEAT TOLERANCE

The mean of the ambient treatment was 12.8 C.

2.8 TREE KELP (PTERYGOPHORA CALIFORNICA) SPOROPHYTE 96-HOUR MEDIAN LETHAL TEMPERATURE (96-H LT50)

Short-term laboratory study of the thermal tolerance of the RIS tree kelp (Pterygophora californica) sporophytes was undertaken in order to determine the upper temperature threshold beyond which the average field-collected plant could not survive. Additionally, information regarding the spontaneity or latency of the species' response to elevated temperatures was sought.

For a review of the literature on tree kelp refer to PGandE (1979b). For the purposes of this experiment it is important to note that the sporophytic generation is the dominant canopy-forming stage in the life-history of the species and that it forms extensive subtidal beds in Diablo Cove and the surrounding area. The tree kelp canopy is believed to be important in maintaining suitable environmental conditions for understory assemblages of flora and fauna.

The study objective was to determine the short-term heat tolerance of tree kelp. The purpose was to provide a basis for an estimation of the effects of the power plant thermal discharge on tree kelp sporophytes in Diablo Cove.

2.8.1 METHODS

A summary description of laboratory methods employed in the investigations into heat tolerance of tree kelp sporophytes is presented below. For a more detailed description of the laboratory methods see APPENDIX B (Procedure 316) and protocol see APPENDIX C (Experiment 16a). Standard methodologies of studying thermal tolerance have not been developed for macroscopic marine algae. To study the thermal tolerance of tree kelp we adopted a 96-hour test period which is a generally accepted period for testing thermal tolerance of marine animals. Determination of death in plants is more difficult to define than it is in animals, because small portions of a plant that survive treatment may in time regenerate an entire new plant. For this reason it was necessary to include in the methodology for this species a post-treatment period during which the plants are returned to ambient temperatures for 96 hours to observe any reversal of temporary effects.

Prior to experiment initiation several tree kelp plants were collected and brought into the laboratory for preliminary testing. These tests demonstrated that brief (less than 60-minute) exposure to water temperatures of 26, 28 and 30 C caused irreversible damage (extensive bleaching, lack of turgor) to the thallus. Tissue samples from these heat-treated thalli were used to develop vital staining techniques best suited to this species (see below).

Selection of test temperatures (ambient, 16, 19, 21 and 24 C) followed from preliminary testing: knowing 26 C to be lethal, 24 C was chosen as the highest temperature which some plants might survive for 96 hours. A value of 16 C represents a warm water season ambient temperature not uncommon in Diablo Cove. The intermediate temperatures (19 and 21 C) were chosen to be equally spaced between 16 and 24 C, and laboratory ambient running seawater provided a "control" test treatment. Hourly temperature data were recorded for each treatment tank by a computer.

Approximately 150 individuals of average total length 225 mm were collected on 30 March 1979 from the area just south of the Diablo Canyon Power Plant southeast breakwater in 25 ft of water. Divers carefully scraped holdfasts from the substrate, placed the plants in collecting bags and upon exiting the water transported the plants (immersed in seawater) directly to the laboratory; there the plants were held for 48 hours in a 15 x 3 x 2 ft raceway supplied with flowing ambient seawater (for a more detailed description of the algal raceways see APPENDIX A). During this time the plants were examined and sorted. Injured or otherwise experimentally unfit individuals were discarded.

Prior to experiment initiation each plant was measured, tagged and photographed. Tags consisted of numbered 10 mm-wide bands of latex surgical tubing stretched over the holdfast and slipped onto the stipe. Plant numbers were haphazardly assigned.

Illumination was provided by banks of 25 W Duro-Test Ultra-H Output Vita-Lights suspended above the raceways. Experimental light intensities were maintained between 121 and 180 μ E/m^2/s for all treatments. A 24-hour (continuous) photoperiod was used.

Twenty plants were introduced into each experimental raceway and twenty additional plants were held at ambient temperatures to serve as a control group. Five remaining plants were designated to be used in initial vital staining observations.

Prior to placement into the experimental raceways the following observations were made and recorded for each plant: bleaching of blade, disintegration of blade stipe and holdfast, mucilage production (normal/abnormal) and thallus turgor. Attempts were made to roughly quantify each of these characters in terms of the degree of response. Plants in each raceway were divided by tag number into four subgroups of five individuals each. At 24-hour intervals for 96 hours one group of five plants from each raceway was sampled for the above-mentioned characteristics (bleaching, disintegration, etc.), photographed and transferred from the experimental (programmed) raceway to an ambient raceway where they were held for 96 hours. Plants were again sampled at the end of this 96-hour "recovery period" (in one case, this period was extended to 120 hours).

Microscopic vital staining techniques were employed in assessing response to temperature at the cellular level. At each observation time one plant from one group of five in each raceway was haphazardly selected and examined (see FIGURE 2.8-1). A small (2 x 2 mm) piece of tissue was excised from a meristematic region and stained with neutral red (MC/B Labs Norwood Ohio) in a solution of 0.3 g/100 ml dH_2O. A wet whole-mount was then prepared from one-half of the stained sample and observed in surface section under a light microscope. Characters observed included color of vacuole, visibility of chloroplasts, presence or absence of plasmolysis and staining of intracellular bodies. Representative fields were photographed at 400x and 1000x. The second half of the stained sample was preserved in 2 percent formalin in seawater and kept in a cool dark place. This was to serve as a back-up sample if required.

TEMPERATURE (C)

ROW	11.7 (AMBIENT)					16.2					19.1					21.0					23.8				
A	01	02	03	04	05	21	22	23	24	25	41	42	43	44	45	61	62	63	64	65	81	82	83	84	85
B	06	07	08	09	10	26	27	28	29	30	46	47	48	49	50	66	67	68	69	70	86	87	88	89	90
C	11	12	13	14	15	31	32	33	34	35	51	52	53	54	55	71	72	73	74	75	91	92	93	94	95
D	16	17	18	19	20	36	37	38	39	40	56	57	58	59	60	76	77	78	79	80	96	97	98	99	100

Numbers represent actual plant identification numbers. Plants in row A were sampled and removed to ambient raceway at 24 hrs; those in row B at 48 hrs; row C, 72 hrs; row D, 96 hrs. Circled numbers indicate plants sampled for vital staining.

FIGURE 2.8-1

DIAGRAM OF PLANT SAMPLES:
TREE KELP HEAT TOLERANCE

At the close of the 96-hour recovery period a second tissue sample was excised from a comparable meristematic region on each plant previously sampled and observed according to the above procedure.

2.8.2 RESULTS

The temperature data for the experiment are summarized in TABLES 2.8-1 and 2.8-2. Taking an approximate mean value of the two probes in each tank, the approximate mean temperatures for the treatment tanks were 16.1, 19.1, 21.0 and 23.8 C and for the first 96 hours of the ambient tank, 11.7 C.

2.8.2.1 BLEACHING

Bleaching of individual thalli was visually estimated and scored as follows: 0 = none, 1 = less than 25 percent of blade area, 2 = less than 50 percent, 3 = less than 100 percent and 4 = total bleaching. Scores for all five plants in each sample group were subsequently averaged to give the values shown in FIGURE 2.8-2. Observations for all five plants at the 96-hour observation were averaged to give values shown in FIGURE 2.8-3. When blade deterioration was observed, bleaching was recorded as 100 percent. With the exception only of the plants held at ambient temperatures, bleaching of the thallus was observed in at least some plants in all treatments (see FIGURES 2.8-2 and 2.8-3). Plants at 23.8 C exhibited bleaching of 65 percent of the blade within 24 hours and total bleaching of the blade area was bleached by 48 hours increasing to 35 percent at 72 hours and 40 percent at 96 hours. Plants held at 19.1 C and 16.2 C did not exhibit bleaching until the 96-hour observation (at 19.1 C 25 percent of the blade area was bleached while only 5 percent was bleached at 16.2 C).

Upon removal from treatment raceways and placement into the ambient recovery raceway, bleaching increased. For the 21.0 C treatment group bleaching of the blade increased from 40 percent at the 96-hour observation period to 75 percent after 96 hours at ambient. Similarly for the 19.1 C treatment group, bleaching increased from 25 percent to 40 percent and for the

TABLE 2.8-1

TREE KELP SPOROPHYTE HEAT TOLERANCE:
SUMMARY TEMPERATURE (C) DATA FOR TREATMENT TANKS

Target Temperature (C)	Treatment Period (H)	Probe	Mean (C)	Standard Deviation	Maximum (C)	Minimum (C)
16	0-24	A	16.0	0.05	16.1	16.0
	0-48	A	16.0	0.05	16.1	16.0
	0-72	A	16.0	0.05	16.1	16.0
	0-96	A	16.1	0.05	16.1	16.0
	0-24	B	16.3	0.05	16.3	16.2
	0-48	B	16.3	0.05	16.3	16.2
	0-72	B	16.3	0.05	16.4	16.2
	0-96	B	16.3	0.05	16.4	16.2
19	0-24	A	19.0	0.05	19.0	18.9
	0-48	A	19.0	0.04	19.0	18.9
	0-72	A	19.0	0.04	19.0	18.9
	0-96	A	19.0	0.04	19.0	18.9
	0-24	B	19.2	0.02	19.3	19.2
	0-48	B	19.2	0.02	19.3	19.2
	0-72	B	19.2	0.03	19.3	19.1
	0-96	B	19.2	0.03	19.3	19.1
21	0-24	A	20.9	0.04	21.0	20.9
	0-48	A	20.9	0.04	21.0	20.9
	0-72	A	20.9	0.04	21.0	20.9
	0-96	A	20.9	0.04	21.0	20.9
	0-24	B	21.2	0.03	21.2	21.1
	0-48	B	21.1	0.03	21.2	21.1
	0-72	B	21.1	0.03	21.2	21.1
	0-96	B	21.1	0.04	21.2	20.9
24	0-24	A	23.8	0.06	24.0	23.8
	0-48	A	23.8	0.04	24.0	23.8
	0-72	A	23.8	0.04	24.0	23.8
	0-96	A	23.8	0.04	24.0	23.8
	0-24	B	23.9	0.06	24.0	23.8
	0-48	B	23.9	0.06	24.0	23.8
	0-72	B	23.9	0.06	24.0	23.8
	0-96	B	23.9	0.06	24.0	23.8

TABLE 2.8-2

TREE KELP SPOROPHYTE HEAT TOLERANCE:

SUMMARY TEMPERATURE (C) DATA FOR AMBIENT (CONTROL AND RECOVERY) TANK

Period (H)	Probe	Mean (C)	Standard Deviation	Maximum (C)	Minimum (C)
0 - 96	A	11.6	0.42	12.6	11.0
97 - 120	A	11.8	0.32	12.5	11.5
121 - 144	A	11.7	0.41	12.4	11.1
145 - 168	A	11.6	0.43	12.3	11.1
169 - 192	A	11.4	0.37	12.0	10.9
193 - 216	A	10.8	0.19	11.1	10.4
0 - 96	B	11.8	0.41	12.8	11.2
97 - 120	B	11.9	0.28	12.6	11.5
121 - 144	B	11.8	0.42	12.6	11.2
145 - 169	B	11.8	0.42	12.5	11.2
169 - 192	B	11.6	0.38	12.2	11.1
193 - 216	B	11.0	0.16	11.2	10.6

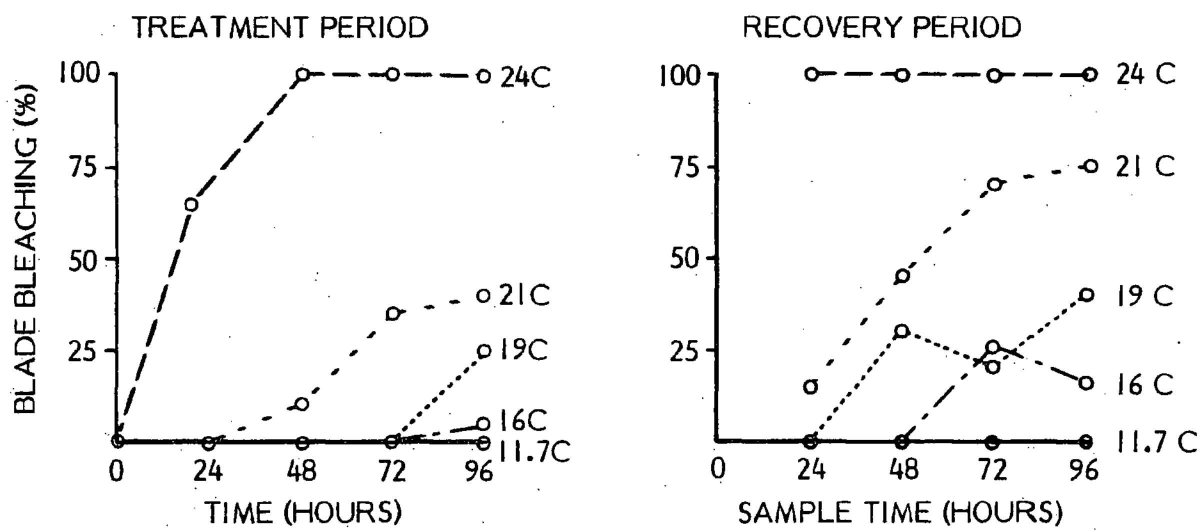

FIGURE 2.8-2

BLADE BLEACHING VERSUS TIME FOR TREATMENT AND RECOVERY PERIODS: TREE KELP SPOROPHYTE HEAT TOLERANCE

The mean temperature of the ambient tank was 11.7 C.

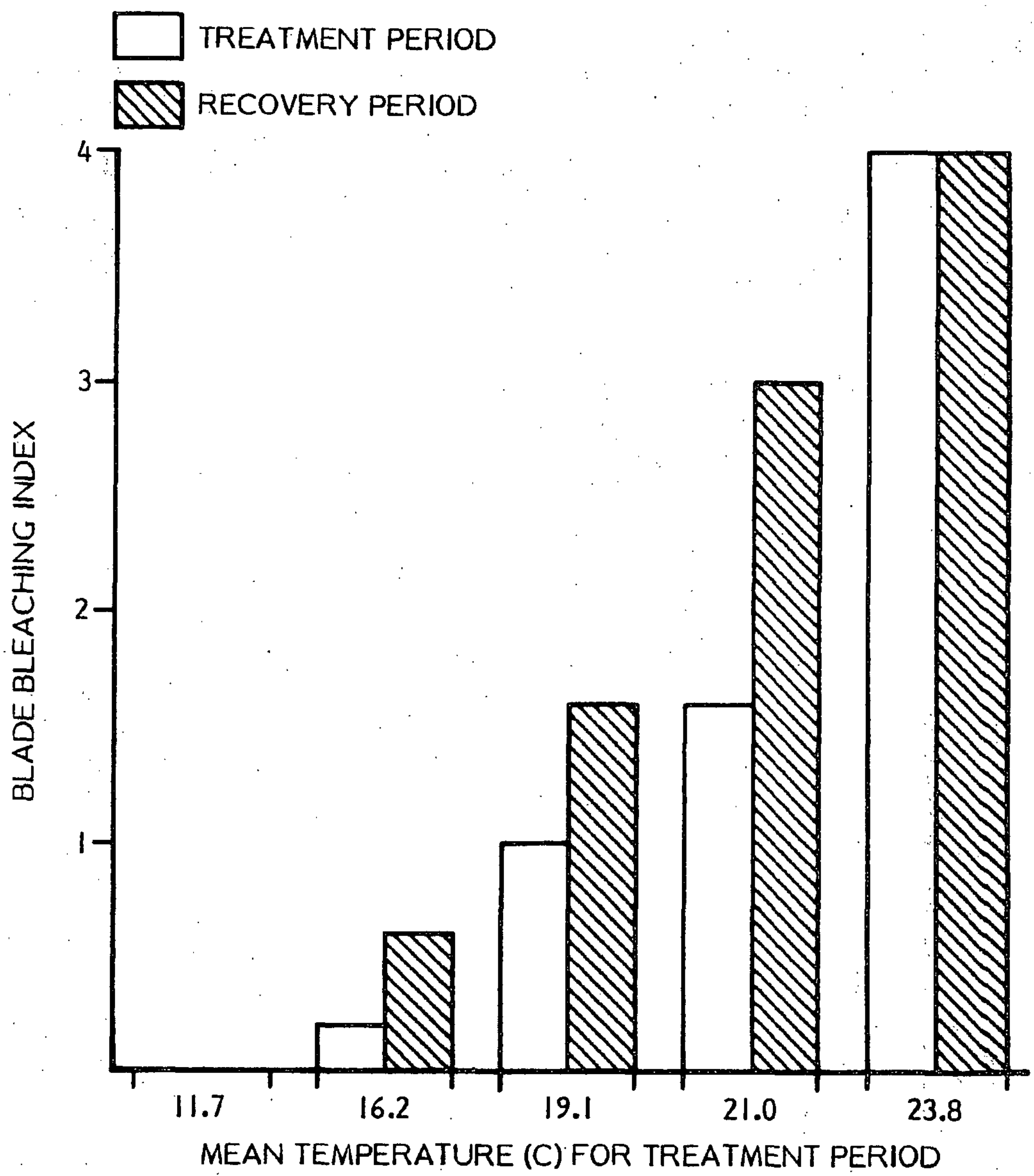

FIGURE 2.8-3

BLADE BLEACHING INDEX VERSUS TEMPERATURE AT THE ENDS OF THE TREATMENT AND RECOVERY PERIODS: TREE KELP SPOROPHYTE HEAT TOLERANCE

The mean temperature of the ambient tank was 11.7 C.

16.2 C group from 5 percent to 15 percent. Control plants held at ambient throughout the experiment showed no bleaching at any time.

2.8.2.2 MUCILAGE

At each observation period blade surfaces were inspected for increase or decrease in mucilage production simply by the observer rubbing the thallus between his fingers. Meristematic regions of fresh health field specimens typically are slimy to the touch owing to the production of small amounts of mucilage by the actively dividing meristematic cells. Copious amounts of mucilage are not commonly produced.

Mucilage production observations for each plant were recorded in the following manner: normal production was scored as "0", and lack of production was scored as "2." The scores were then averaged for all five plants in each sample group to obtain values plotted in FIGURE 2.8-4. Scores for all five plants at the 96-hour observation were averaged giving the values seen in FIGURE 2.8-5. When complete blade deterioration occurred, mucilage production was scored as "2."

Meristematic regions of plants held at elevated temperatures were found to be considerably less "slimy" to the touch than those of freshly-collected or ambient-held plants. As can be seen in FIGURE 2.8-4, plants at 23.8 C produced no discernable meristematic mucilage after 24 hours; plants at 21.0 C stopped producing mucilage at 48 hours and those at 19.1 C terminated mucilage production after 96 hours. Plants at 16.2 C exhibited a variable response through time but the magnitude of this response was much smaller than that of plants in higher temperature treatments and will be discussed further below. Ambient plants showed no decrease in mucilage production throughout the 96-hour experimental period.

Upon transfer to the ambient recovery raceway, recovery (resumption of mucilage production) occurred in the 16.2, 19.1 and 21.0 C treatment plants. In the 16.2 C treatment, recovery was complete in those groups that had experienced a decrease in mucilage production (48- and 96-hour groups). In the 19.1

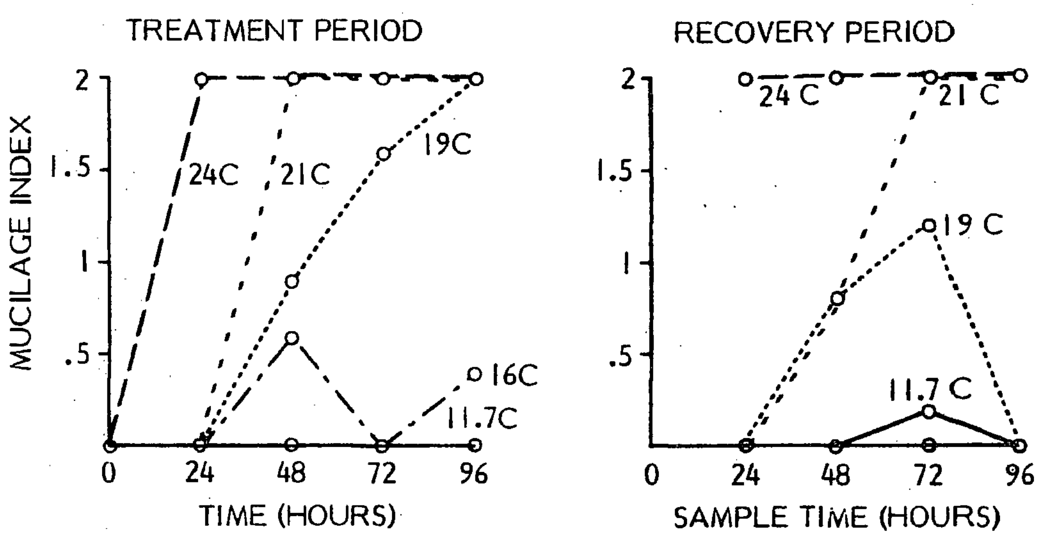

FIGURE 2.8-4

MUCILAGE INDEX VERSUS TIME FOR THE TREATMENT
AND RECOVERY PERIODS:
TREE KELP SPOROPHYTE HEAT TOLERANCE

A higher mucilage index value indicates a greater thermal effect.
The mean temperature of the ambient tank was 11.7 C.

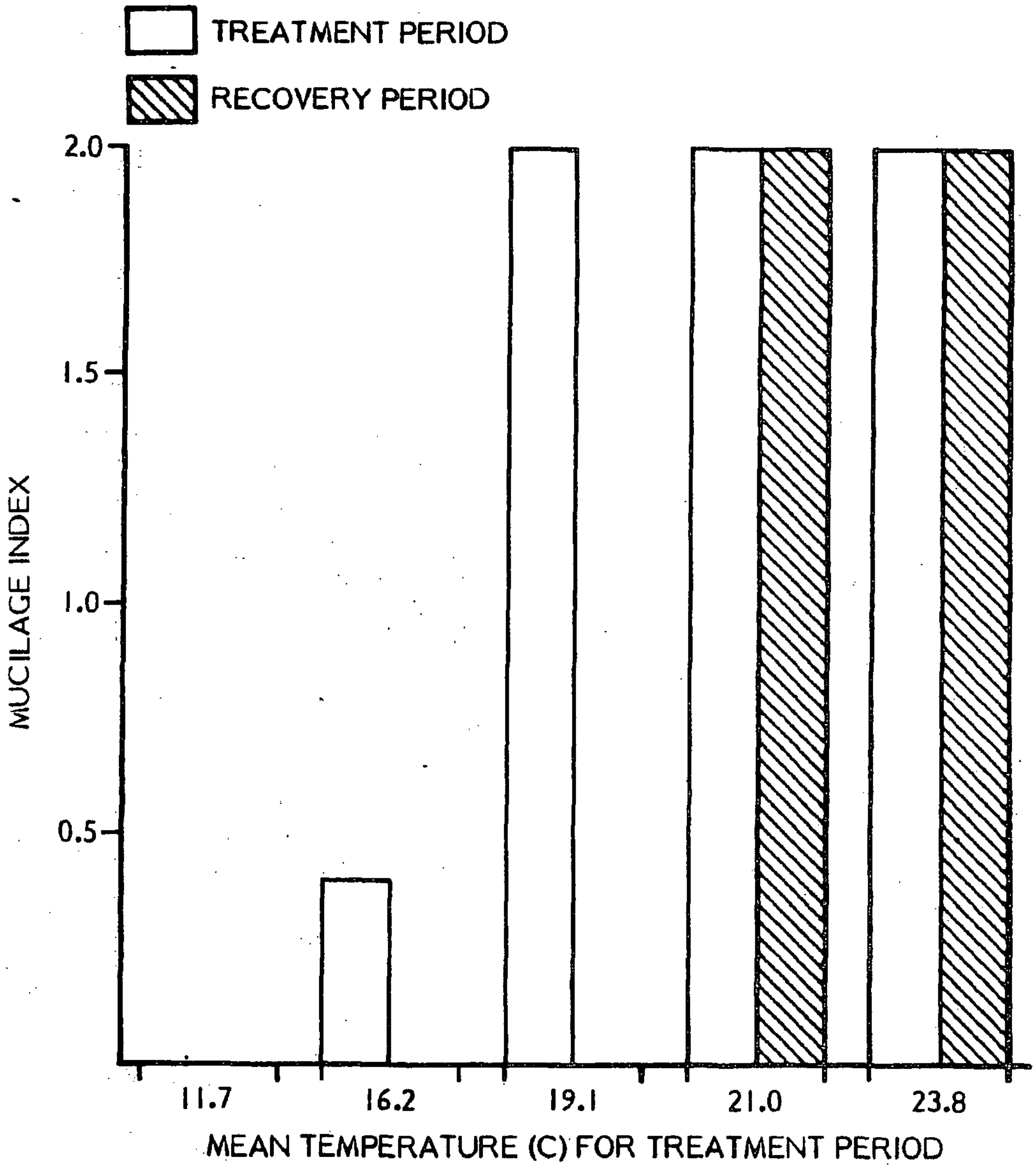

FIGURE 2.8-5

MUCILAGE INDEX VERSUS TEMPERATURE AT THE ENDS
OF THE TREATMENT AND RECOVERY PERIODS:
TREE KELP SPOROPHYTE HEAT TOLERANCE

A higher mucilage index value indicates a greater thermal effect.

The mean temperature of the ambient tank was 11.7 C.

treatment there was slight recovery of the 48-hour group, considerable recovery of the 72-hour group, and complete recovery of the 96-hour group. Of the 21.0 C treatment plants, only the 48-hour group showed recovery (about half of the plants resumed mucilage production).

A slight decrease in mucilage production was observed in the 72-hour sample of the ambient treatment after transfer to the recovery raceway; this is of comparatively slight magnitude and will be discussed below.

FIGURE 2.8-5 reiterates data shown in FIGURE 2.8-4 with the exclusion of time as a variable. The histogram depicting results for the experimental period clearly shows a decrease in mucilage production with increased temperature. Diminished values for the 19.1 C treatment for the recovery period should be noted.

2.8.2.3 TURGOR

Thallus turgor observations were scored as "0" (indicating normal turgor) or "1" (indicating lack of turgor). Observations were subsequently averaged for all 20 individuals within a treatment. When blade deterioration was observed, turgor was scored as "1." Lack of thallus turgor increased with increasing temperature (FIGURE 2.8-6). The maximum response (complete lack of turgor) was elicited at 23.8 C in both experimental and recovery periods. Plants held at 21.0 C exhibited lack of turgor to a lesser extent in both experimental and recovery periods; at 19.1 C two plants showed lack of turgor after 120 hours in the recovery raceway.

2.8.2.4 BLADE DISINTEGRATION

Blade disintegration was visually estimated and scored as follows: 0 = none, 1 = less than 25 percent of blade area, 2 = less than 50 percent, 3 = less than 100 percent and 4 = total bleaching. After 96 hours at test temperatures only plants in the 23.8 C treatment had undergone any blade deterioration; in this group disintegration continued after transfer to the ambient recovery raceway

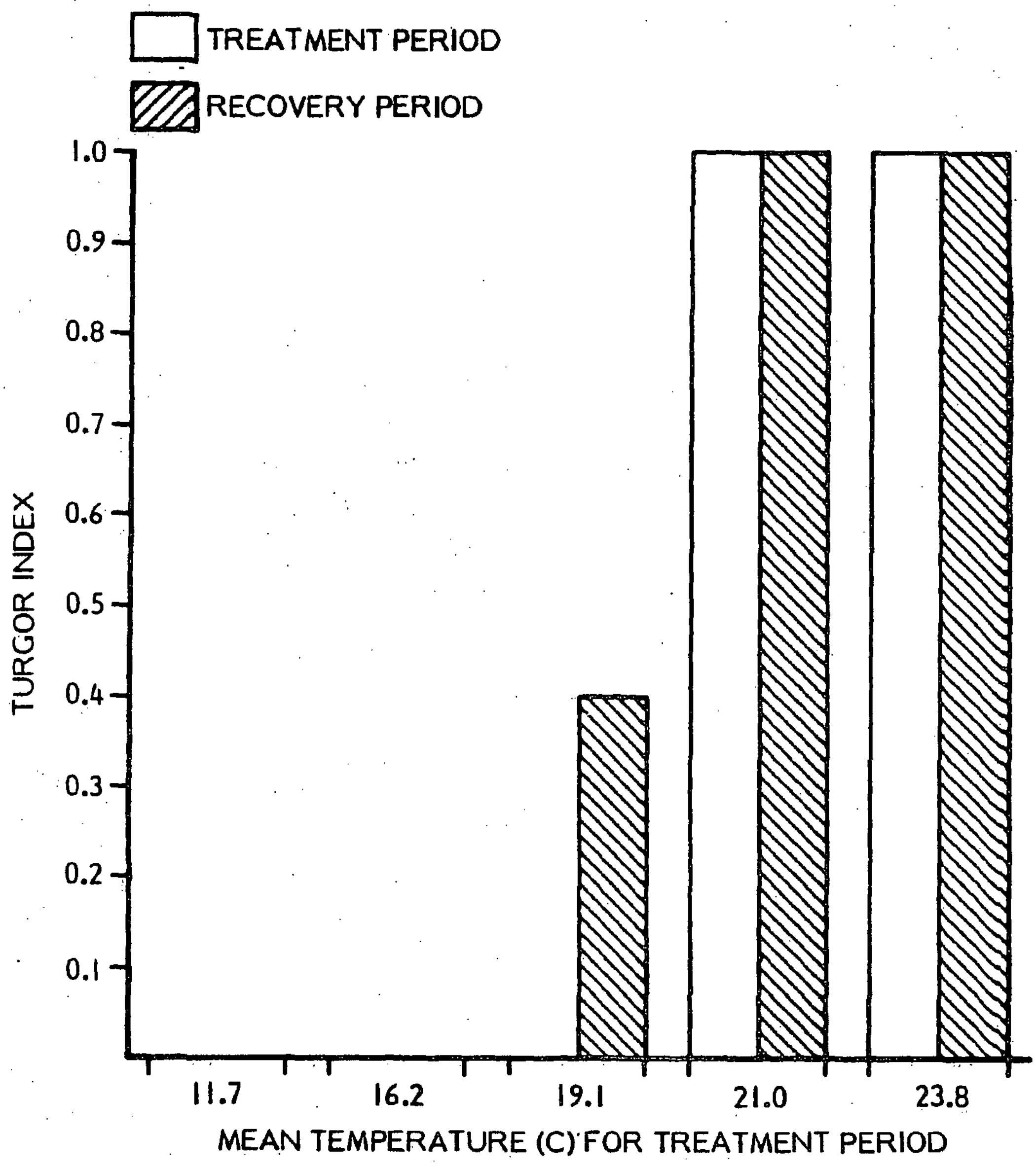

FIGURE 2.8-6

TURGOR INDEX VERSUS TEMPERATURE AT THE ENDS OF THE TREATMENT AND RECOVERY PERIODS: TREE KELP SPOROPHYTE HEAT TOLERANCE

A higher turgor index value indicates a greater thermal effect.

The mean temperature of the ambient tank was 11.7 C.

until, after 120 hours at ambient, disintegration amounted to nearly 90 percent of the total blade area (FIGURE 2.8-7).

A much smaller amount of disintegration was seen in the 21.0 C plants after 120 hours in the recovery raceway though no disintegration was found while the plants were held in the treatment raceways.

2.8.2.5 STIPE/HOLDFAST DISINTEGRATION

Stipe and holdfast disintegration were scored as: 0 = none, 1 = partial and 2 = complete. The data presented in FIGURE 2.8-8 represent the mean values for the five plants of the 96-hour sample. Disintegration of stipes and holdfasts was of small magnitude occurring only at 23.8 C at 96 hours in the experimental period and at 72 and 120 hours in the recovery period. This response may be attributable to localized degeneration at higher temperatures of wounds sustained during collecting and may indicate a lack of wound-healing capacities at temperatures above 21.0 C.

2.8.2.6 VITAL STAINING

Results obtained from vital staining investigations are somewhat more difficult to interpret than the results discussed above. All observations pertaining to injury are comparative and based upon typical staining patterns of fresh healthy material or conversely, intensely (30 C) heat-treated material. Vital staining observations of color were scored as follows: 0 = normal, 0.5 = partial thermal injury and 1.0 = total thermal injury. The data presented in FIGURES 2.8-9 to 2.8-11 represent observations on a single plant in each treatment at the end of the 96-hour period.

The color of stained vacuoles is indicative of the capacity of the cells to actively take up stain: dead cells lack this capacity. FIGURE 2.8-9 is a graph of differences in vacuolar color as a function of temperature. In the experimental period, plants at ambient showed normal uptake of stain, those at 16.2 C were moderately affected and those at 19.1, 21.0 and 23.8 C exhibited complete

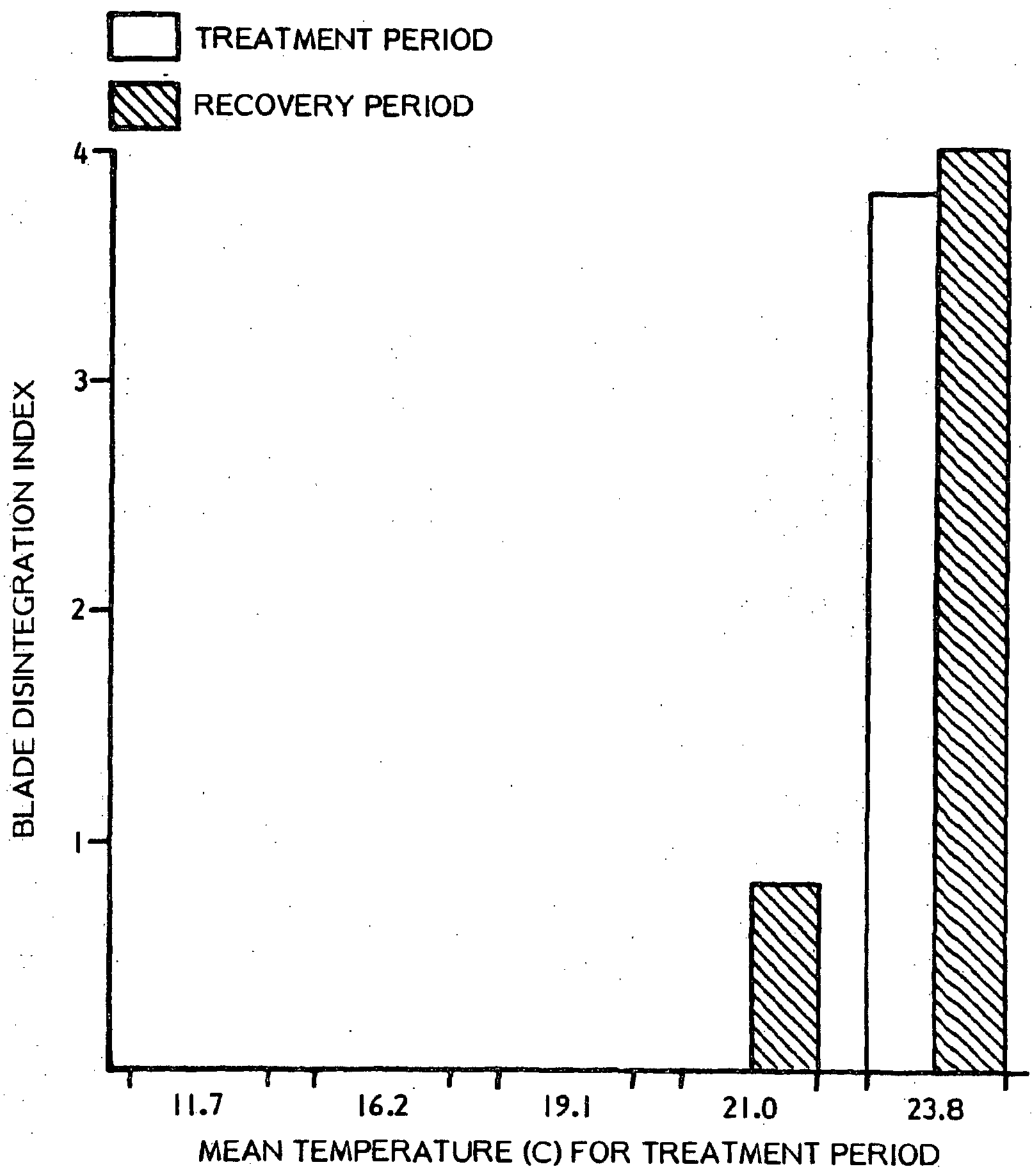

FIGURE 2.8-7

BLADE DISINTEGRATION INDEX VERSUS TEMPERATURE AT THE ENDS OF THE TREATMENT AND RECOVERY PERIODS: TREE KELP SPOROPHYTE HEAT TOLERANCE

The mean temperature of the ambient tank was 11.7 C.

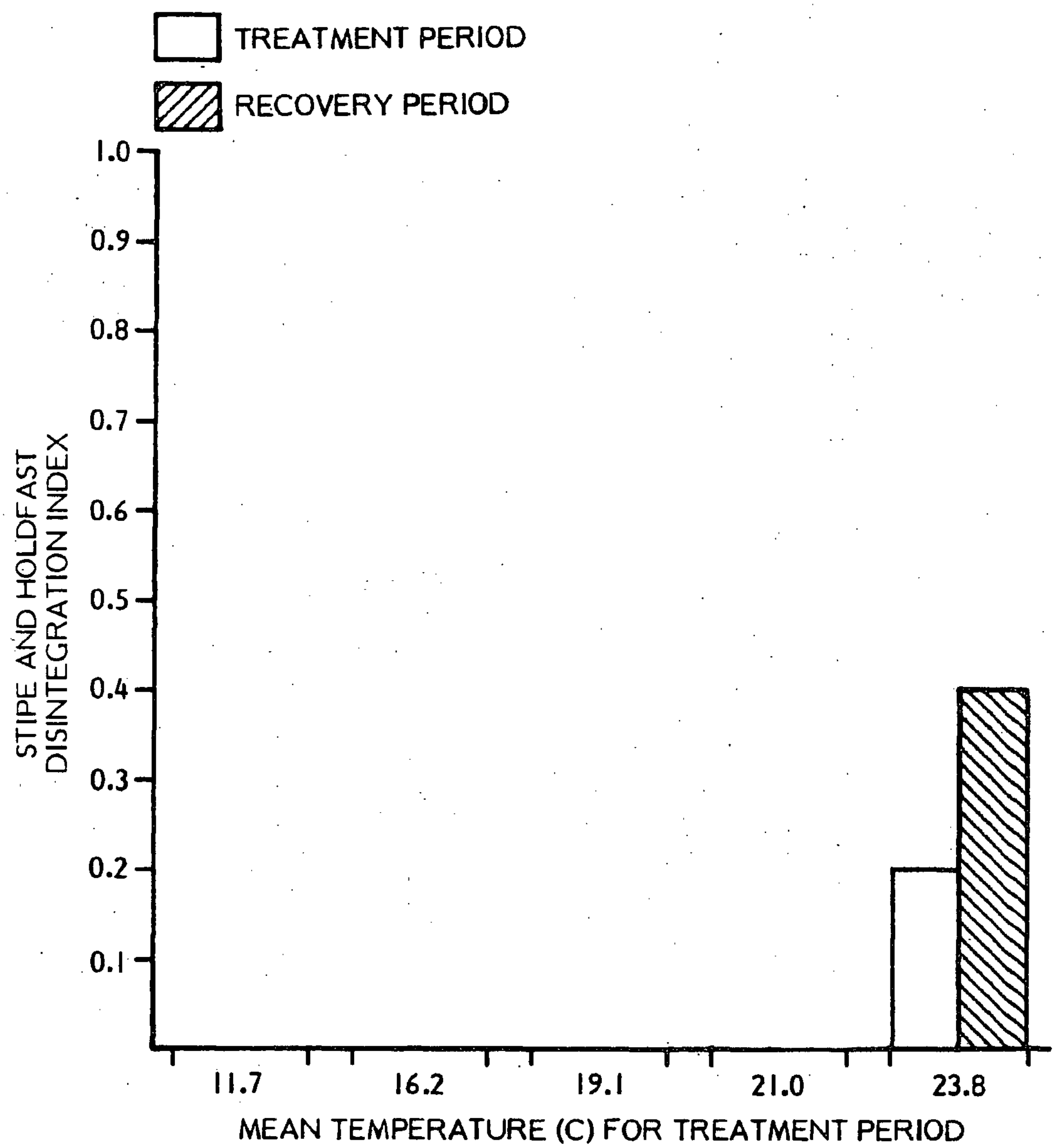

FIGURE 2.8-8

STIPE AND HOLDFAST DISINTEGRATION INDEX VERSUS TEMPERATURE AT THE ENDS OF THE TREATMENT AND RECOVERY PERIODS:
TREE KELP SPOROPHYTE HEAT TOLERANCE

The mean temperature of the ambient tank was 11.7 C.

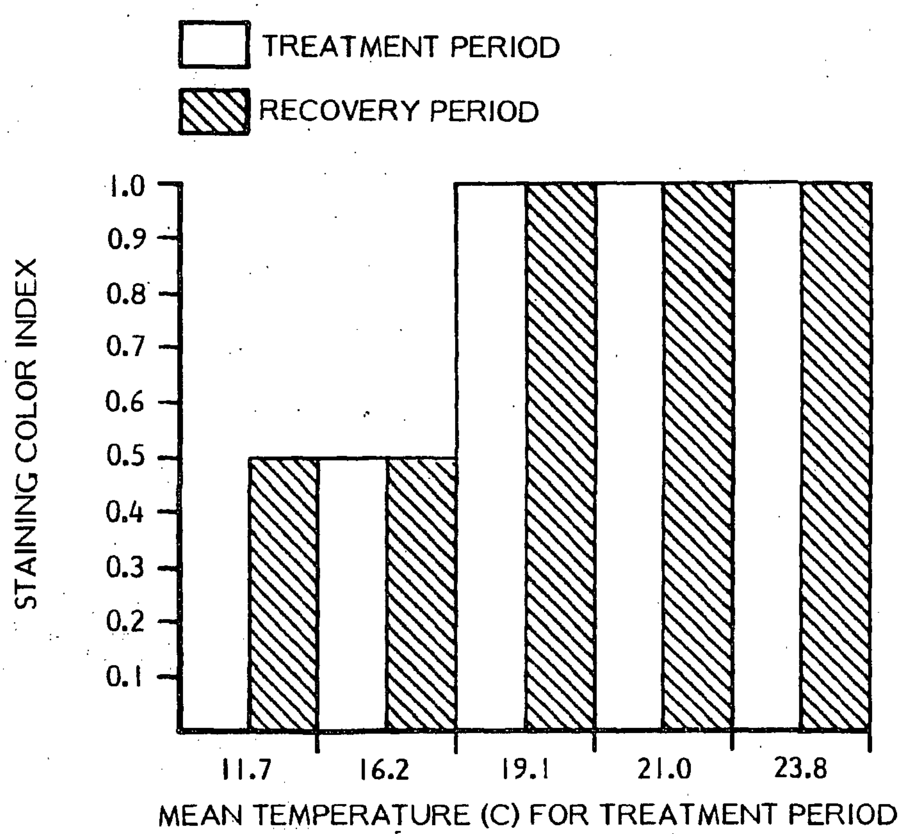

FIGURE 2.8-9

STAINING COLOR INDEX VERSUS TEMPERATURE AT THE ENDS OF THE TREATMENT AND RECOVERY PERIODS: TREE KELP SPOROPHYTE HEAT TOLERANCE

A higher staining color index value indicates more thermal damage. The mean temperature of the ambient tank was 11.7 C. Values based on a visual scan of one plant at each observation.

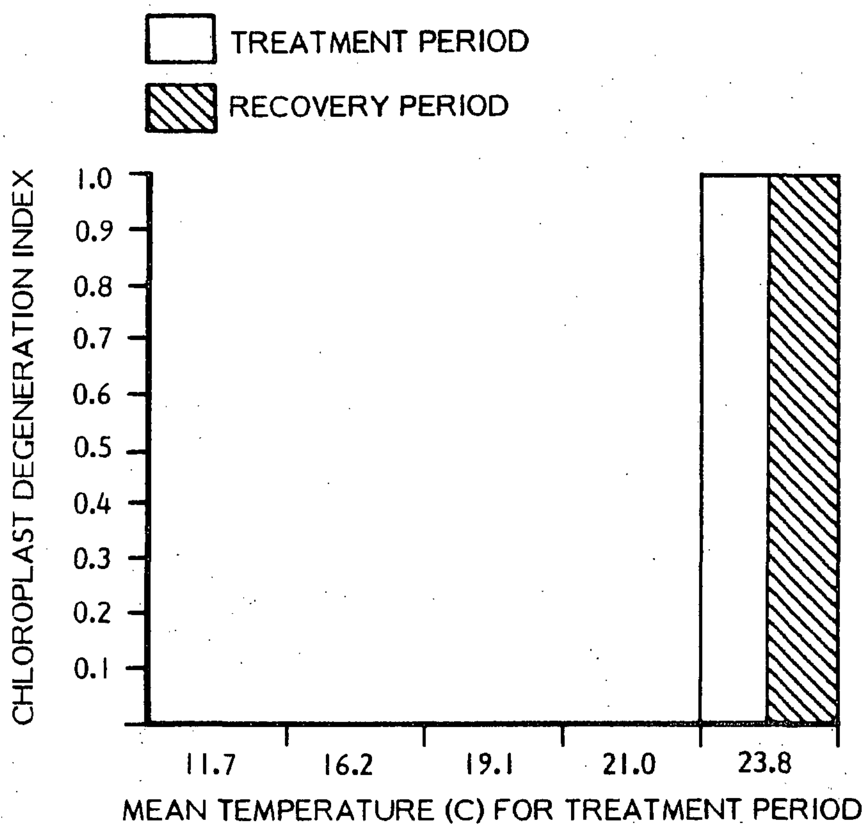

FIGURE 2.8-10

CHLOROPLAST DEGENERATION INDEX VERSUS TEMPERATURE
AT THE ENDS OF THE TREATMENT AND RECOVERY PERIODS:
TREE KELP SPOROPHYTE HEAT TOLERANCE

A higher chloroplast degeneration index value indicates more thermal damage. The mean temperature of the ambient tank was 11.7 C. Values based on a visual scan of one plant at each observation.

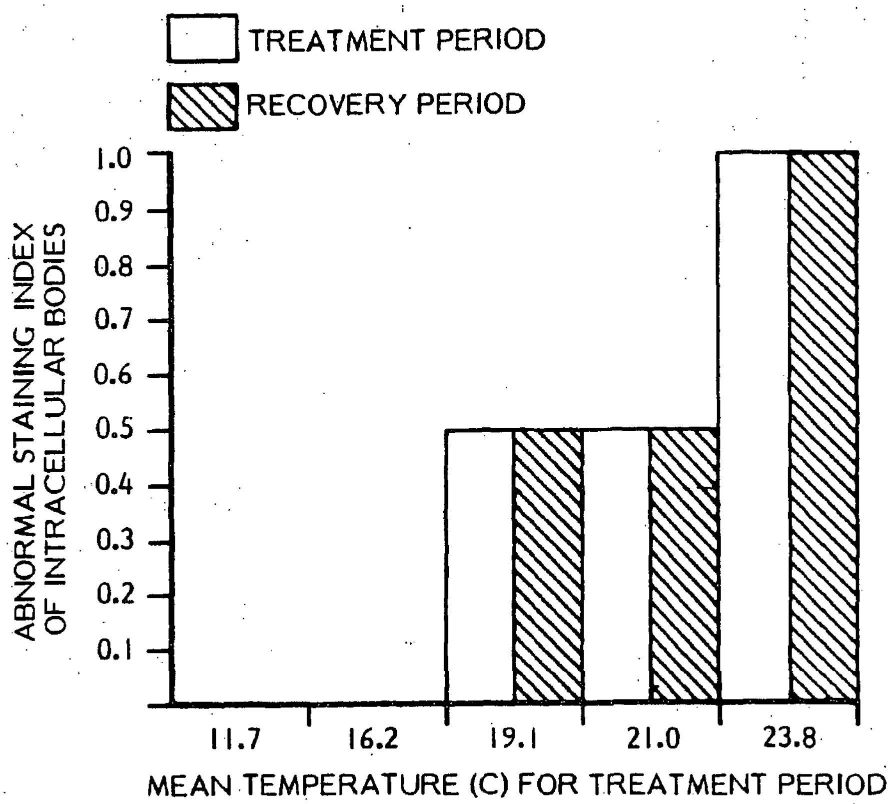

FIGURE 2.8-11

ABNORMAL STAINING INDEX OF INTRACELLULAR BODIES VERSUS TEMPERATURE AT THE ENDS OF THE TREATMENT AND RECOVERY PERIODS: TREE KELP SPOROPHYTE HEAT TOLERANCE

A higher staining index value indicates greater abnormality in staining characteristics. The mean temperature of the ambient tank was 11.7 C. Values based on a visual scan of one plant at each observation.

abnormal staining. Only one change was noted in the recovery period: abnormal staining appeared in the ambient plant.

In each stained sample, chloroplasts were observed for discernable signs of injury. In only the 23.8 C treatment was chloroplast damage found (FIGURE 2.8-10).

Intracellular bodies (probably starch granules) are common in the blade tissue cells of tree kelp and were found to be excellent indicators of cellular stain uptake. Dark red staining of these bodies is typical for fresh health material and all ambient (control) treatment tissue samples exhibited such staining. As indicated in FIGURE 2.8-11 staining of intracellular bodies was normal at ambient and 16.2 C, intermediate at 19.1 and 21.0 C and completely abnormal at 23.8 C.

2.8.3 DISCUSSION

Based on the character of blade bleaching, plants of tree kelp are adversely affected by temperature in proportion to the time of exposure. Plants experiencing 23.8 C (74.8 F) for 48 hours will be killed, assuming that 100 percent bleaching is equivalent to death. Exposure to 21.0 C (69.8 F) results in 40 percent of the blade surface bleaching at 96 hours; to 19.1 C (66.4 F), 25 percent of the blade area; and to 16.2 C (61.2 F), 5 percent of the blade area. Using a recovery period following exposure to the experimental conditions, bleaching was observed to increase markedly following the treatment period. Judging by the results of this experiment, significant mortality in tree kelp populations would be expected following exposure to 19.1 C (66.4 F) or higher for 96 hours or more. Thus it would appear that any increase in temperature above the maximum warm season temperatures normally recorded in Diablo Cove (16 C = 61 F) will have some effect upon tree kelp populations. These results were generally confirmed by vital staining analysis. The importance of changes in turgor and mucilage production, which generally followed the trends in blade bleaching, are difficult to assess with respect to survivorship in nature.

Though only 5 percent of blade surface was bleached after 96 hours at 16.2 C (61.2 F), an increase in bleaching occurred in the 72-hour treatment group in the ambient recovery raceway indicating as in the 19.1 C (66.4 F) and 21.0 C (69.8 F) treatments latent injury response to temperature.

2.10 OTHER SPECIES

2.10.1 BLACK SURFPERCH (EMBIOTOCA JACKSONI)

According to Fitch and Lavenberg (1971), the black surfperch, Embiotoca jacksoni, occurs in loose schools in sandy and rocky habitats along the outer coast and in bays, usually shallower than 24 m (80 ft) depth. Its geographical distribution extends from Fort Bragg, California to Punta Abreojos, Baja California, Mexico (Miller and Lea 1972). It reaches a maximum length of 30 cm (15 in.).

Although black surfperch is not an RIS and has not been recorded in the 316(a) samples as one of the more abundant fishes, it is present in the Diablo Cove vicinity and some populations of this species could be exposed to the thermal plume.

The results of 96-hour median lethal temperature and critical thermal maximum experiments are presented in this section.

2.10.1.1 96-HOUR MEDIAN LETHAL TEMPERATURE (96 H LT50)

The purposes of experiments on the heat tolerance of juvenile black surfperch were the following:

- To observe the time course of mortality for groups of fish exposed to heated water over a 96-hour period;
- To determine from these observations the temperatures at which 50 percent of the fish died (LT50 values) for standarized 96-hour exposures and for shorter exposures to heated water;
- To measure the effect of acclimation to warm (16C) and cold (12.2C) temperatures on the LT50 values for the fish.

The acclimation temperatures were selected to represent average winter (12C) and summer (16C) water temperatures in the Diablo Canyon area, in order to assess the effect of seasonal ambient water temperatures on the species' thermal tolerance.

Methods

A summary descripton of the laboratory methods employed in the investigations of heat tolerance (LT50 determination) of black surfperch is presented in SECTION 2.1.1.1 of this report. For a more detailed description of the laboratory methods see APPENDIX B (Procedure 305) and protocols see APPENDIX C (experiments 5a to d). Juvenile black surfperch were collected using a 3 m (loft) otter trawl during July and August 1978, from an area in the outer region of Morro Bay. The fish were immediately transferred to the thermal effects laboratory at Diablo Canyon and held in ambient temperature seawater prior to acclimation. Holding tanks were raised or lowered 1C per day until the proper acclimation temperature (12C or 16C) was reached. The fish were then held at this temperature until acclimated.

The length of acclimation period was determined by the use of critical thermal maximum tests (CTM) to monitor changes in the fish thermal tolerance levels. In the CTM tests four fish were removed from the acclimation tank and placed in a small aquarium with an immersion heater. The temperature in the tank was raised at a constant rate of approximately 7C per hour, and the temperature at which the fish expired was recorded (the mean temperature at mortality of four fish equals the CTM). Theoretically, a fish acclimating to a warmer temperature should exhibit an increasing tolerance to elevated temperatures and, thus, a higher CTM. The rate of increase in CTM temperatues levels off when the fish are fully acclimated to the new temperature (a matter of hours or days depending on the species and the temperature differential involved). The minimum acclimation period during the 96-hour LT50 experiment was 6 days.

Following acclimation, 10 individuals were transferred to each of six experimental tanks and held for 12 to 24 hours at their acclimation temperature to allow them to adjust to their new surroundings. At the start of the experiment, one tank remained at the acclimation temperature while the other five tanks were raised to their various elevated test temperatures, within one hour if possible (some of the warmer tanks required a slightly longer period for temperature adjustment, but all temperatures were reached within 2 hours). Observations were made every hour for the first six hours, and following this period the fish were observed at 18, 30, 42, 54, 66, 78, 90 and 96 hours. Dead fish were removed and measured, and behavioral notes were made as part of the observations. Following the 96-hour period the tanks were returned to the acclimation temperature, and a final set of observations was made 24 hours later. The experiments were repeated twice for fish from each of the two acclimation temperatures. The first experiment involved a wider range of test temperatures than the second and the results were used to select a narrower range of temperatures used in the second experiment. The time between experiments ranged from 1-8 days.

Test temperatures were controlled and recorded on a hourly basis by an Autodata Nine temperature controlling and data acquisition system described in APPENDIX A. Mean experimental temperatures were calculated from hourly readings for the 96-hour test period or for that period leading up to 100 percent mortality of a group of fish. When the time to 100 percent mortality was substantially shorter than 96 hours, the variability of the test temperature was strongly influenced by the initial temperature adjustment period and resulted in relatively higher standard deviations about the mean. A failure of the seawater system for 3 hours during hours 22-25 of the second experiment on 12C acclimated fish (Experiment 5d) resulted in a maximum 2C temporary decrease in the test temperature. However, in spite of this mishap, the results of this experiment were in close agreement with the other experiment on 12C acclimated fish (Experiment 5c).

Probit analysis of the mortality data was carried out in accordance with procedures specified in Standard Methods (1976).

Results

Juvenile black surfperch acclimated to 12.2C and 16.0C were exposed to various test temperatures ranging form 23.9C to 27.5C, and the time courses of mortality during the two experiments on each acclimation group are shown in TABLES 2.10.1-1 and 2.10.1-2. For each observation period, cumulative mortalities were plotted on a probability scale versus their respective mean test temperatures. Example plots are shown in FIGURE 2.10.1-1 for 66 and 78 hour observations of the 16C acclimated fish, and FIGURE 2.10.1-2 for the 6 and 18 hour observations of the 12.2C acclimated fish. A line fitted by eye to each of the plots was used to extrapolate the temperature at which exactly 50 percent mortality of each group of fish would occur. In each acclimation group, the extrapolated temperatures which caused 50 percent mortality were then plotted for each observation period (FIGURE 2.10.1-3). The temperature at which 50 percent mortality (LT50) occurs for any length of exposure up to 96 hours can be estimated graphically from the handplotted curves for each group. The 96-hour LT50 for juvenile black surfperch acclimated to 16.0C was 25.6C, and the 96-hour LT50 for the 12.2C acclimated group was 24.5C, about 1C lower. For the 12.2C acclimated group, exposures to 24.5C for periods longer than about 12 hours did not produce any additional mortality. This temperature (24.5C) is the estimated "upper incipient lethal temperature" for the 12.2C acclimated group. Black surfperch that were acclimated to 16C, tolerated warmer temperatures for longer periods than fish acclimated to 12.2C, but the 96-hour experimental period was not sufficiently long to define the upper incipient lethal temperature for the 16C acclimated group.

Discussion

Over 96 hours, juvenile black surfperch acclimated to 16C (61F) tolerated higher temperatures than juveniles acclimated to 12.2C (54F). The warm acclimated group had an LT50 of 25.6C (78F), and the cold-acclimated group had an LT50 of 24.5C (76F). Therefore, these fish would be expected to tolerate elevated temperatures better during warm fall periods than during colder summer periods. Temperatures lower than 24.5C (76F), the upper incipinet lethal temperature,

TABLE 2.10.1-1

PERCENTAGE THERMAL MORTALITY
IN 12C-ACCLIMATED JUVENILE BLACK SURFPERCH

Species: Embiotoca jacksoni Acclimation Temperature: 12.2C

$\overline{X}$ Standard Length: 72.5 mm.
$\overline{X}$ Weight: 13.8 g.

Experiment 5c 10-07-78 through 10-11-78

$\overline{X}$ Experiment Temperatures (C):	12.1	24.0	24.6	24.9	25.5	25.9
Standard Deviations:	0.08	0.07	0.04	0.05	0.03	0.14

Experiment 5d 10-20-78 through 10-24-78

$\overline{X}$ Experiment Temperatures (C):	12.1	23.9	24.2	24.4	24.7	25.0
Standard Deviations:	0.15	0.29	0.27	0.25	0.22	0.04

Zero Mortality (C): 12.1, 24.0, 24.2

PERCENTAGE MORTALITY

Experiment Code		5d	5d	5c	5d	5c	5d	5c	5c
Temperature, C		23.9	24.4	24.6	24.7	24.9	25.0	25.5	25.9
Time (hours)	1								10
	2							20	100
	3							50	
	4		10			30	20	80	
	5		20			40	40	90	
	6			10	20				
	18	10	40	40	80	100	100	100	
	30	*	*		*				
	42								
	54				90				
	66								
	78								
	90								
	96		50						
Total Percentage Mortality		10	50	40	90	100	100	100	100

* 3-hour pump failure.

TABLE 2.10.1-2

PERCENTAGE THERMAL MORTALITY
IN 16C-ACCLIMATED JUVENILE BLACK SURFPERCH

Species: *Embiotoca jacksoni* Acclimation Temperature: 16.0C

$\overline{X}$ Standard Length: 69.0 mm.
$\overline{X}$ Weight: 12.3 g.

Experiment 5a 09-10-78 through 09-14-78

$\overline{X}$ Experiment Temperatures, C:	16.0	21.8	23.8	24.7	25.8	27.5
Standard Deviations:	0.00	0.04	0.05	0.06	0.12	0.35

Experiment 5b 09-16-78 through 09-20-78

$\overline{X}$ Experiment Temperatures, C:	16.0	24.9	25.2	25.5	25.7	26.0
Standard Deviations:	0.03	0.06	0.07	0.05	0.17	0.10

Zero Mortality, C: 16.0, 21.8, 23.8, 24.7, 25.2

PERCENTAGE MORTALITY

Experiment Code		5b	5b	5b	5a	5b	5a
Temperature, C		24.9	25.5	25.7	25.8	26.0	27.5
Time (hours)	1						20
	2						90
	3						100
	4						
	5						
	6						
	18			10	10	20	
	30				30	30	
	42					40	
	54					80	
	66					100	
	78			50	50		
	90		30	60	90		
	96	10					
Total Percentage Mortality		10	30	60	90	100	100

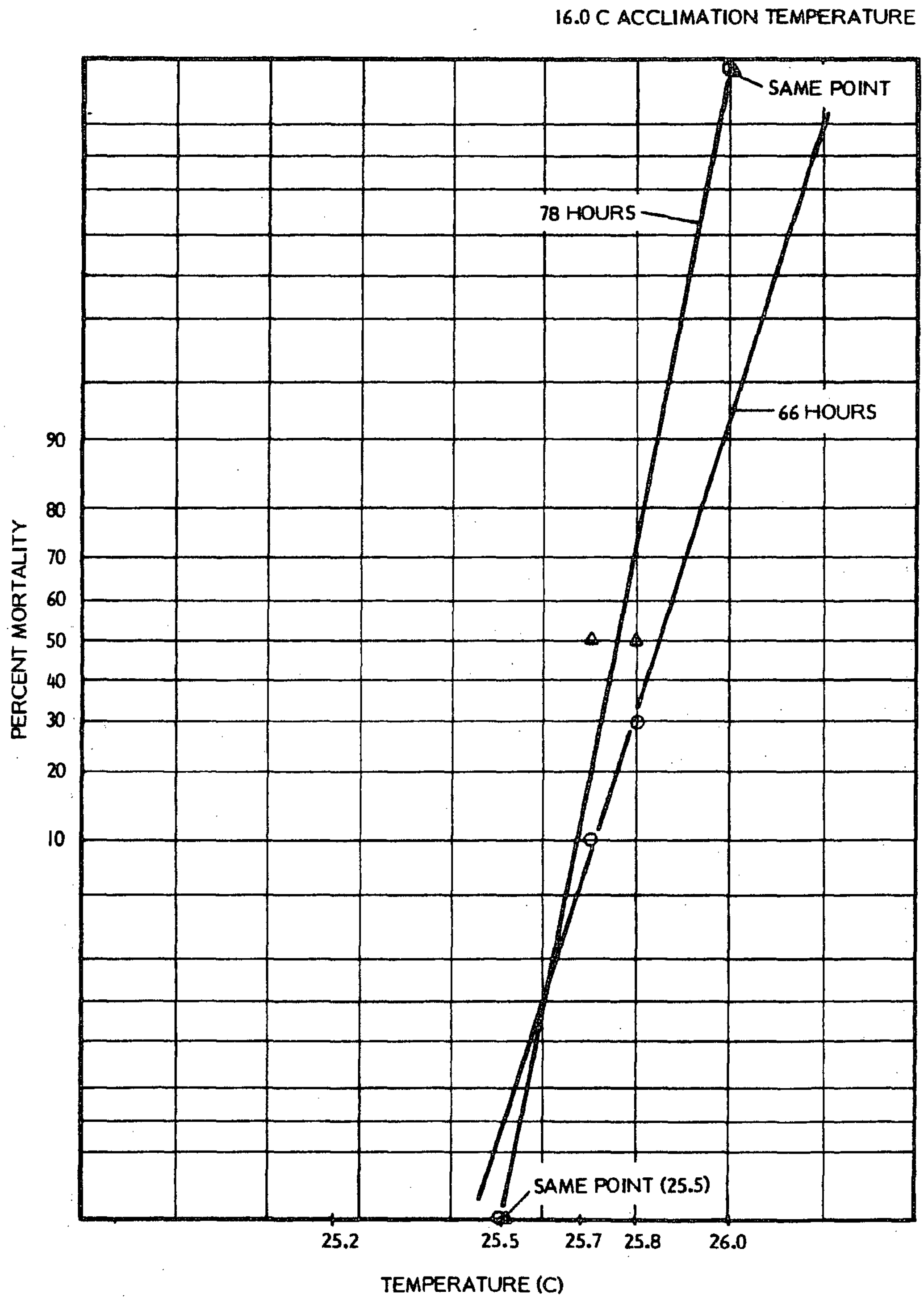

FIGURE 2.10.1-1

PERCENTAGE THERMAL MORTALITY VERSUS TEMPERATURE AT 66 AND 78 h FOR JUVENILE BLACK SURFPERCH ACCLIMATED TO 16 C IN EXPERIMENTS 5a AND b

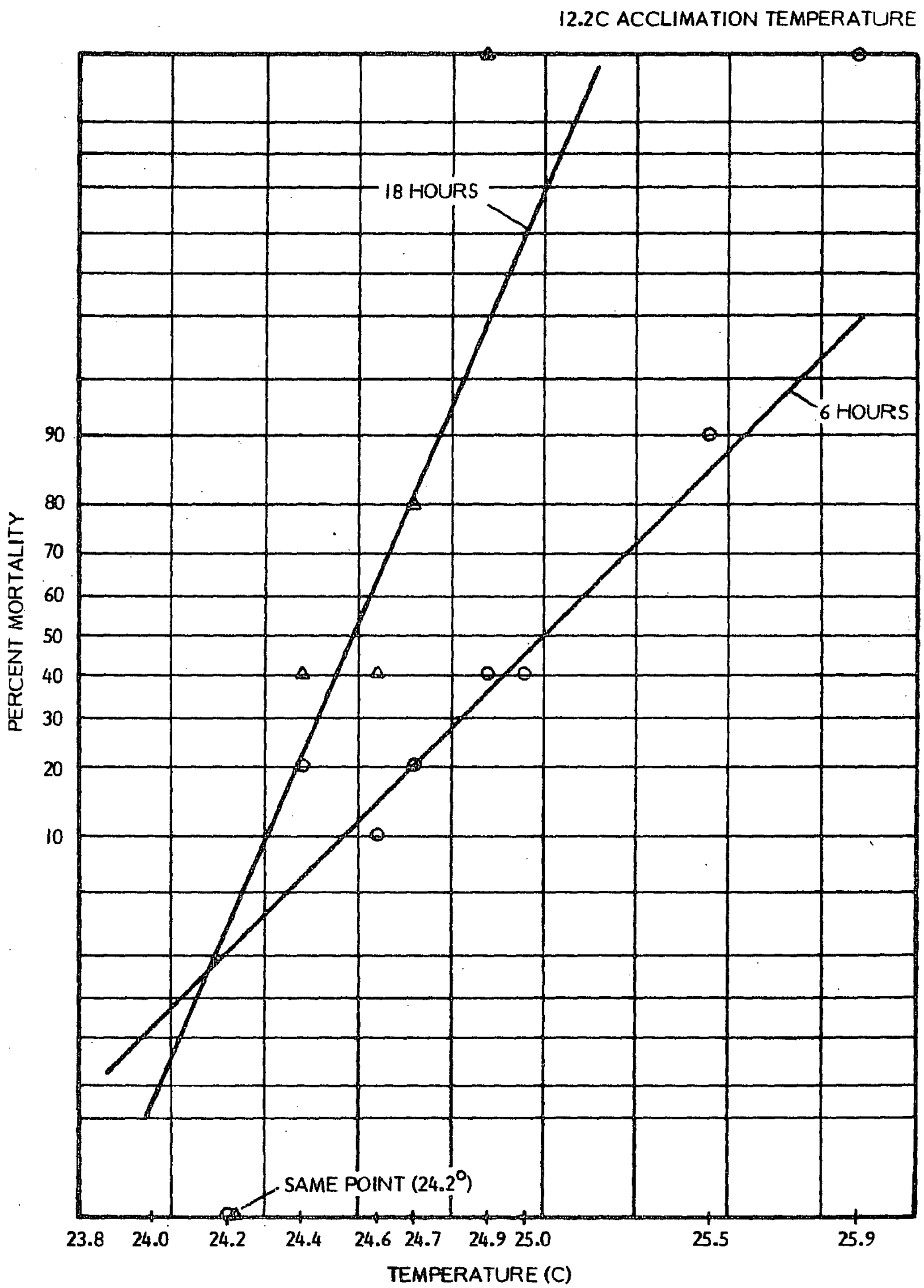

FIGURE 2.10.1-2

PERCENTAGE THERMAL MORTALITY VERSUS TEMPERATURE AT 6 AND 18 h FOR JUVENILE BLACK SURFPERCH ACCLIMATED TO 12.2 C IN EXPERIMENTS 5a AND b

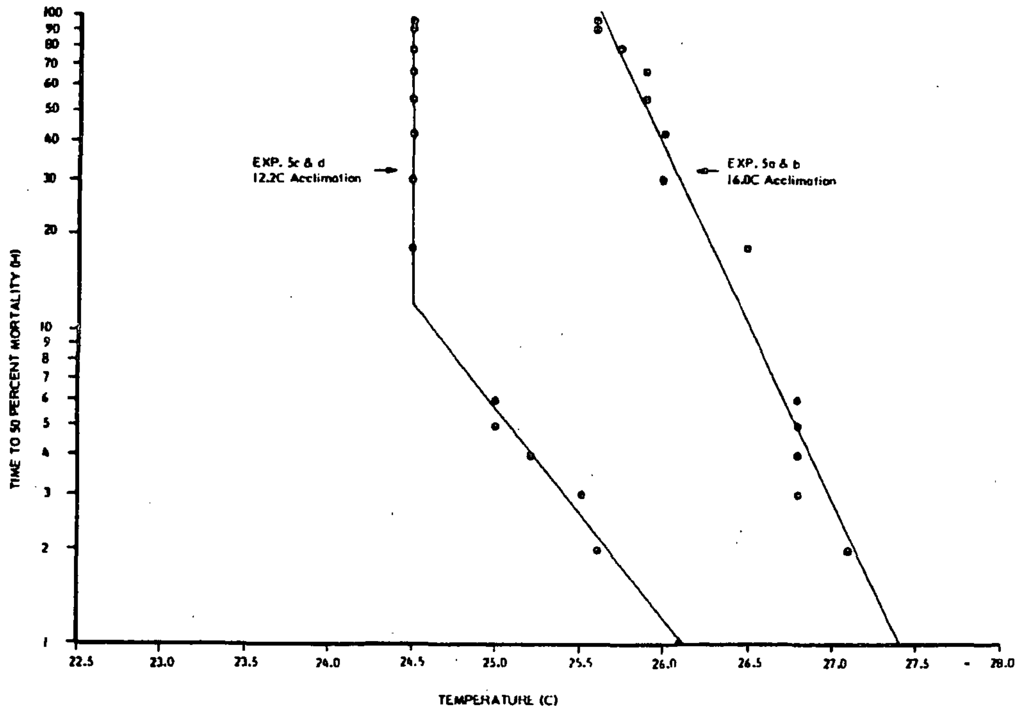

FIGURE 2.10.1-3

TIME TO 50 PERCENT THERMAL MORTALITY VERSUS TEMPERATURE
OF JUVENILE BLACK SURFPERCH ACCLIMATED
TO 12.2 C (EXPERIMENTS 5c AND d) AND 16 C (EXPERIMENTS 5e AND g)

would result in less than 50 percent mortality for the cold-acclimated fish. Although the 16C (61F) acclimated fish showed an increased resistance to elevated temperatures for 96 hours, exposures longer than 96 hours might result in 50 percent or more mortality at temperatures less than 25.6C (78F).

2.10.1.2 CRITICAL THERMAL MAXIMUM (CTM)

The objective of the critical thermal maximum experiment on black surfperch was to provide information on the point of mortality of fishes exposed to a rapidly increasing temperature. This standard test provides an index to a species' heat tolerance and can also be used to determine at what point black-and-yellow/gopher rockfish become acclimated to certain temperatures, in preparation for other experiments. The purpose was to provide information about the possible impact of the thermal plume on the species, in particular, the mortality expected when the black-and-yellow/gopher rockfish suddenly encounters relatively high temperatures from which it cannot escape.

Methods

A summary of the methods employed in the critical thermal maximum experiment on black surfperch was presented in SECTION 2.1.2.1. The protocols for this experiment are included in APPENDIX C of this report (experiments 8j, 8l, 8m, 8n, and 8q). A more detailed account of the experimental procedure used in critical thermal maximum experiments is presented in APPENDIX B (Procedure 308).

The adult specimens of black surfperch used in this experiment were collected between May and early June 1978 from Morro Bay (an area adjacent to the Morro Bay Power Plant intake structure) by beach seine and otter trawl.

The fish were held in 4 x 8 ft flow-through tanks. During the holding period, the fish were carefully observed and those which appeared to be injured or otherwise unhealthy were excluded from use in the experiment.

Temperatures in the tanks were controlled (where required) and recorded by an Autodata Nine computer. The temperature data were transcribed from paper tapes onto data sheets and summarized as cumulative means and standard deviations for 24-hour intervals during the holding period.

Immediately prior to each run, the test tank (see description in SECTION 2.1.2.1) was filled with seawater at the holding temperature of the test population. The fish were then transferred to the test tank and allowed to adjust to the new surroundings for 15-60 minutes. After the adjustment period, heating of the water commenced at the rate of 0.1C per minute. The fish were initially observed at 15-minute intervals. The temperature of the tank and comments relating to the condition of the fish were recorded at each observation. As the fish appeared to show signs of severe stress (disorientation and/or loss of equilibrium, depending on the species), observations were made continuously and upon the death of each individual (defined by cessation of opercular movement) the time, number of deaths and temperature of the water were recorded until all of the fish in a test population had succumbed. The mean value of the temperatures recorded at death represented the critical thermal maximum value for that test.

Because of the versatility of the experiment, it was used to test populations of fish subjected to different holding conditions: ambient (fluctuating) temperatures and acclimating to a constant controlled temperature.

A one-way ANOVA test was used to detect significant differences between runs. If significant differences were detected, a posteriori comparisons were performed using Duncan's new multiple range test to determine which of the runs differed.

Results

A total of five CTM experiments, comprising from three to eight runs, were conducted on this species. Each run involved four fish. The runs were made both on fish held at ambient temperatures and on fish acclimating to controlled temperatures. Summary descriptions of the five experiments follow:

- experiment 8j: eight runs, first run 17 July 1978, acclimating to 16C (raised from ambient, approximately 11C, at 1C/day), runs after 0, 2, 4, 7, 10, 14, 16, and 18 days at 16C

- experiment 8l: three runs (24, 28 and 29 August 1978), held at ambient temperatures (approximately 11C at the time of CTM testing)

- experiment 8m: three runs (25, 28 and 29 August 1978), held at ambient temperatures (approximately 11C at the time of CTM testing), held separately from those in experiment 8l

- experiment 8n: four runs, first run 6 September 1978, acclimating to 16C (raised from ambient, approximately 13C, at 1C/day), runs after 1, 2, 3, and 4 days at 16C

- experiment 8q: five runs, first run 28 September 1978, acclimating to 12C (lowered from ambient, approximately 12.8C, at 1C/day), runs after 1, 4, 5, 8, and 9 days at 12C.

The data from all of these runs are presented in TABLES 2.10.1-3 to 2.10.1-7.

Discussion

Significant differences were detected in CTM values of black surfperch acclimating to 16C (61F) in experiment 8j. Specifically, CTM values recorded 10 or more days after acclimation began were significantly higher than those at seven or fewer days after acclimation began. In a similar experiment (8n), no significant differences were detected in CTM values during the first four days of acclimation to 16C (61F).

TABLE 2.10.1-3

SUMMARY DATA AND STATISTICAL RESULTS: BLACK SURFPERCH CRITICAL THERMAL MAXIMUM EXPERIMENT 8J

SUMMARY DATA

Run	Mean Length (cm)	Mean Weight (g)	No. Fish	Mean CTM (C)	Days At 16C
1	11.3	53.4	3[a]	28.2	0
2	10.8	44.6	4	28.0	2
3	11.5	54.7	4	28.2	4
4	11.0	48.8	4	27.9	7
5	11.5	52.4	4	28.8	10
6	10.5	39.7	4	29.1	14
7	11.6	52.6	4	29.2	16
8	11.7	53.8	4	29.2	18
Overall Mean	11.2	49.9	(31)	28.6	--

STATISTICAL RESULTS

ANOVA: $F_{(7,23)} = 6.788$ (significant at 0.01 level)

Duncan's Test (bars connect runs not significantly different)

Days at 16C	7	2	0	4	10	14	16	18
CTM (C)	27.9	28.0	28.2	28.2	28.8	29.1	29.2	29.2

[a] One fish was found to be damaged. Its data were excluded from this table.

NOTE: The fish were held at ambient temperatures (11-12C on the day preceding temperature control initiation) prior to holding at 16C.

TABLE 2.10.1-4

SUMMARY DATA AND STATISTICAL RESULTS: BLACK SURFPERCH CRITICAL THERMAL MAXIMUM EXPERIMENT 8L.

SUMMARY DATA

Run	Mean Length (cm)	Mean Weight (g)	No. Fish	Mean CTM (C)	Days
1	6.6	8.3	4	28.5	1
2	6.3	8.1	3[a]	27.9	4
3	6.1	7.2	4	27.6	5
Overall Mean	6.3	7.8	(11)	28.0	--

STATISTICAL RESULTS

ANOVA: $F_{(2,8)} = 43.5$ (significant at 0.01 level)

Duncan's Test (bars connect runs not significantly different)

CTM (C)	27.6	27.9	28.5

[a] Data from one premature death were excluded.

NOTE: The fish were held at ambient temperatures (12-15C) prior to and during the experiment.

TABLE 2.10.1-5

SUMMARY DATA AND STATISTICAL RESULTS: BLACK SURFPERCH CRITICAL THERMAL MAXIMUM EXPERIMENT 8M

SUMMARY DATA

Run	Mean Length (cm)	Mean Weight (g)	No. Fish	Mean CTM (C)	Days
1	7.0	10.8	4	28.5	1
2	7.2	12.1	4	28.4	4
3	7.2	12.6	4	28.4	5
Overall Mean	7.1	11.8	(12)	28.4	--

STATISTICAL RESULTS

ANOVA: $F_{(2,9)} = 6.9098$ (not significant at 0.01 level)

NOTE: The fish were held at ambient temperatures (12-15C) prior to and during the experiment.

TABLE 2.10.1-6

SUMMARY DATA AND STATISTICAL RESULTS: BLACK SURFPERCH CRITICAL THERMAL MAXIMUM EXPERIMENT 8N

SUMMARY DATA

Run	Mean Length (cm)	Mean Weight (g)	No. Fish	Mean CTM (C)	Days at 16C
1	6.3	8.0	4	28.5	1
2	6.4	8.0	4	28.7	2
3	6.8	11.1	4	29.0	3
4	7.0	11.3	4	29.2	4
Overall Mean	6.6	9.6	(16)	28.8	--

STATISTICAL RESULTS

ANOVA: (not significantly different at 0.01 level)

NOTE: The fish were held at ambient temperatures (approximately 13C) prior to holding at 16C.

TABLE 2.10.1-7

SUMMARY DATA AND STATISTICAL RESULTS: BLACK SURFPERCH
CRITICAL THERMAL MAXIMUM EXPERIMENT 8Q

SUMMARY DATA

Run	Mean Length (cm)	Mean Weight (g)	No. Fish	Mean CTM (C)	Days at 12C
1	7.5	15.2	4	28.2	1
2	8.0	16.1	4	27.9	4
3	6.8	10.5	4	27.9	5
4	7.5	13.8	4	27.6	8
5	7.6	14.0	4	28.1	9
Overall Mean	7.5	13.9	(20)	27.9	--

STATISTICAL RESULTS

ANOVA: (not significantly different at 0.01 level)

NOTE: The fish were held at ambient temperatures (12.5-13C) prior to holding at 12C.

No significant differences were detected in CTM values of black surfperch acclimating to 12C (54F) over a period of nine days. This result was not unexpected because the ambient holding temperature of these populations was close to the temperature of acclimation.

Of the CTM tests conducted on black surfperch held at ambient temperatures, all runs within an experiment were not significantly different except for runs 2 and 3 of experiment 81, which appeared to give significantly lower readings than run 1. It is possible that variations in the ambient holding temperature during the experiment could have caused the difference observed.

CTM values of black surfperch are influenced by holding temperature in that higher holding temperature results in higher CTM value after a period of time.

2.10.2 OLIVE ROCKFISH (SEBASTES SERRANOIDES) 96-HOUR MEDIAN LETHAL TEMPERATURE (LT50)

The olive rockfish (Sebastes serranoides) is not one of the RIS and experiments had not been planned on this species. However, recent studies (PGandE 1979a) showed that juveniles of this species comprise a large portion of observed fish on transects in Diablo Cove. When large numbers of juveniles were netted together with blue rockfish (S. mystinus) it was decided to run a simultaneous 96-hour LT50 experiment on the olive rockfish because this would not require a significant increase in effort. Sufficient numbers of animals were obtained only for one acclimation temperature: 16 C.

Olive rockfish range from the San Benito Islands off Baja California, Mexico to Redding Rock in Del Norte County, California. They have been reported from the surface down to a depth of 146 m (480 ft) (Miller and Lea 1972). Olive rockfish are commonly found in close association with kelp beds along the central coast of California. Adults, in the vicinity of Diablo Canyon, are usually observed as solitary individuals although they can occur in large groups. Juvenile olive rockfish are at times quite numerous and accounted for 12.4 percent of all the fish observed along transect lines in Diablo Cove from 1976 through December 1978 (PGandE 1979a). Both juveniles and adults usually occupy the midwater portion of the water column, either below the Macrocystis/Nereocystis canopy or just above beds of smaller macroalgae (Ebeling and Bray 1976). This species frequently schools with blue rockfish (Sebastes mystinus, an RIS).

A gut content analysis study of olive rockfish off Santa Barbara by Love (1974) revealed that adults of this species fed primarily on fish. Zooplankton and canopy dwelling invertebrates made up the remainder of the diet. It would appear from this study that feeding is confined to free living (as opposed to attached) organisms found in the kelp canopy and midwater depths. It should be noted that juvenile rockfish, including olive rockfish, were part of the adult diet.

Development in olive rockfish, as in all rockfish of the genus Sebastes, is ovoviviparous. Fertilization is internal. Copulation is believed to occur before ovulation, with sperm stored in the ovary until needed (Miller et al. 1967). Spawning occurs from December through March. A large female may release up to 650,000 larvae during spawning (Frey 1971).

The study objectives of the experiment on median lethal temperature of juvenile olive rockfish were the following:

- To observe the time course of mortality for groups of fish exposed to heated water over a 96-hour period
- To determine from these observations the temperatures at which 50 percent of the fish died (LT50 values) for standardized 96-hour exposures and for shorter exposures to heated water

The acclimation temperature was selected to represent average warm season (16 C) water temperature in the Diablo Canyon area. The purpose of this study was to determine the effect of the power plant discharge on juvenile olive rockfish acclimated to warm season temperatures.

2.10.2.1 METHODS

Juvenile olive rockfish were collected using a 12 x 12 ft diver-operated lift net. The collections were made in the PGandE Diablo Canyon power plant intake cove (that area enclosed by the two jetties) during June 1979.

Methods used for these experiments were identical to those described in SECTION 2.1.1.1. The temperature records and methods of data analysis were also the same. A more detailed description of the methods used is included in APPENDIX B (Procedure 305). The protocol for this run of the experiment (5o) is included in APPENDIX C.

2.10.2.2 RESULTS

Juvenile olive rockfish acclimated to 16 C were exposed to various test temperatures ranging from 20.0 to 25.8 C. The time course of mortality is shown in TABLE 2.10.2-1. For each observation period, cumulative mortalities were plotted on a probability scale versus their respective mean test temperatures. A line fitted by eye to each of the plots was used to extrapolate the temperature at which exactly 50 percent mortality would occur. The extrapolated temperatures which caused 50 percent mortality were then plotted for each observation period through 96 hours (FIGURE 2.10.2-1). The temperature at which 50 percent mortality (LT50) occurs for any length of exposure up to 96 hours can be estimated graphically from the hand-plotted curve. The 96-hour LT50 for juvenile olive rockfish acclimated to 16 C was 24.4 C.

2.10.2.3 DISCUSSION

For olive rockfish acclimated to warm season (16 C = 61 F) temperatures, no thermal mortality would be expected at temperatures of 23.8 C (74.8 F) or lower for periods of up to four days. About one half of the population might be expected to die at 24.4 C (75.9 F) after a 96-hour exposure.

TABLE 2.10.2-1

OLIVE ROCKFISH HEAT TOLERANCE:

SUMMARY TEMPERATURE AND THERMAL MORTALITY DATA ON 16 C ACCLIMATED ANIMALS

Mean Standard Length: 74 mm
Mean Wet Weight: 7.9 g

Experiment 5o: 08-13-79 through 08-17-79

Mean Experimental Temperatures (C):	16.0	20.0	21.9	23.1	23.8	25.0	25.8
Standard Deviations:	0.05	0.05	0.04	0.01	0.05	0.05	0.05

Zero Mortality (C): 16.0, 20.0, 21.9*, 23.1, 23.8

96 H CUMULATIVE PERCENTAGE MORTALITY

Temperature (C)	25.0	25.8
Hours		
1		20
2	10	80
3		100
4	40	
5	50	
6		
18	100	
30		
42		
54		
66		
78		
90		
96	100	100

* Three fish at 21.9 C became trapped beneath the 2 x 3-ft cage and died as a result. These were not thermally related deaths.

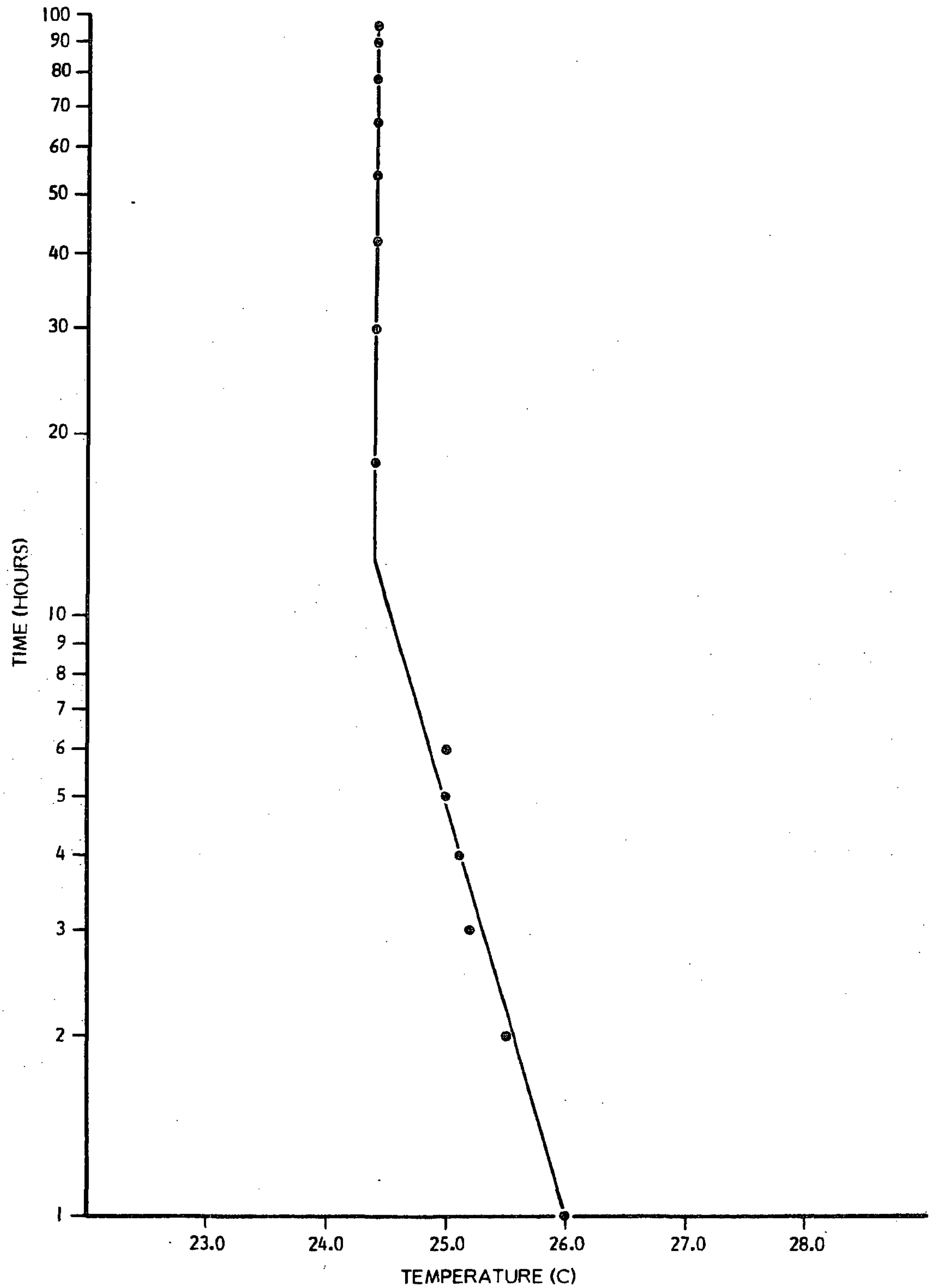

FIGURE 2.10.2-1

TIME TO 50 PERCENT MORTALITY: OLIVE ROCKFISH HEAT TOLERANCE

TIME TO 50 PERCENT MORTALITY:

The animals were acclimated to 16 C (Experiment 5o).

2.10.3 REEF SURFPERCH (MICROMETRUS AURORA)CRITICAL THERMAL MAXIMUM (CTM)

Juvenile populations of the reef surfperch, Micrometrus aurora, occur commonly in schools in nearshore waters around Diablo Canyon. Small schools are often found trapped in tidepools at low tide. This species has a very narrow depth distribution, having been reported from the intertidal down to 6 m (20 ft) (Miller and Lea 1972). Its geographical distribution extends from Tomales Bay, California to Punta Baja, Baja California, Mexico (Miller and Lea 1972). It reaches a maximum length of 18 cm (7 in.).

Although reef surfperch is not an RIS and has not been recorded in the 316(a) samples as one of the more abundant fishes, it is present in the Diablo Cove vicinity and some populations of this species could be exposed to the thermal plume, particularly in view of their shallow water distribution.

The objective of the critical thermal maximum experiment on reef surfperch was to provide information on the point of mortality of fishes exposed to a rapidly increasing temperature. This standard test provides an index to a species' heat tolerance and can also be used to determine at what point black-and-yellow/gopher rockfish become acclimated to certain temperatures, in preparation for other experiments. The purpose was to provide information about the possible impact of the thermal plume on the species, in particular, the mortality expected when the black-and-yellow/gopher rockfish suddenly encounters relatively high temperatures from which it cannot escape.

2.10.3.1 METHODS

A summary of the methods employed in the critical thermal maximum experiments was presented in SECTION 2.1.2.1. The protocol for this experiment is included in APPENDIX C of this report (experiment 8c). A more detailed account of the experimental procedure used in critical thermal maximum experiments is presented in APPENDIX B (Procedure 308).

The specimens of reef surfperch used in this experiment were collected in August 1979 from a tidepool in Field's Cove using hand nets.

The fish were held in 4x8 ft flow-through tanks at ambient temperatures. During the holding period, the fish were carefully observed and those which appeared to be injured or otherwise unhealthy were excluded from use in the experiment.

Immediately prior to each run, the test tank (see description in SECTION 2.1.2.1) was filled with seawater at the holding temperature of the test population. The fish were then transferred to the test tank and allowed to adjust to the new surroundings for 15 to 60 minutes. After the adjustment period, heating of the water commenced at the rate of 0.1C per minute. The fish were initially observed at 15-minute intervals. The temperature of the tank and comments relating to the condition of the fish were recorded at each observation. As the fish appeared to show signs of severe stress (disorientation and/or loss of equilibrium, depending on the species), observations were made continuously and upon the death of each individual (defined by cessation of opercular movement) the time, number of deaths and temperature of the water were recorded until all of the fish in a test population had succumbed. The mean value of the temperatures recorded at death represented the critical thermal maximum value for that test. A one-way ANOVA test was used to detect significant differences between runs.

2.10.3.2 RESULTS

One CTM experiment, comprising six runs, was conducted on this species. Each run involved five fish. The runs were made on fish held at ambient temperatures. Prior to the testing period (February 1980), mean ambient seawater temperature was 14.3C. During the test period (3 to 5 March 1980), daily mean ambient seawater temperatures ranged from 14.5 to 14.9C.

The mean CTM values of the runs ranged from 30.0 to 30.3C and a significant difference between runs was not detected by ANOVA (TABLE 2.10.3-1).

TABLE 2.10.3-1

SUMMARY DATA AND STATISTICAL RESULTS: REEF SURFPERCH CRITICAL THERMAL MAXIMUM EXPERIMENT 8C

SUMMARY DATA

Run	Mean Length (mm)	Mean Weight (g)	Mean CTM (C)
1	61	5.8	30.2
2	62	6.1	30.3
3	65	6.7	30.2
4	67	8.1	30.1
5	64	6.6	30.0
6	62	6.4	30.0
Overall Mean	64	6.5	30.1

STATISTICAL RESULTS

ANOVA: $F_{(5,24)} = 0.547$ (not significant at 0.01 level)

2.10.3.3 DISCUSSION

CTM values for reef surfperch were determined to be relatively high: 30C (86F). This result is not unexpected in view of the fact that the species occurs only in shallow water and is thus subject to relatively high temperatures in the natural habitat.

2.10.4 ACANTHINA SPIRATA 96-HOUR MEDIAN EFFECTIVE TEMPERATURE (96 H ET50)

According to Morris et al. (1980), the angular unicorn, Acanthina spirata, occurs commonly in high and middle intertidal zones on protected rocks and pilings. Its geographical distribution extends from Tomales Bay, Marin County, California to Camalu, Baja California, Mexico. It is one among many murid snail species occurring along the central California coast, with a shell length to 40 mm. The closely related A. punctulata is reported to feed on littorines, barnacles and Tegula funebralis.

Although A. spirata is not an RIS and has not been recorded in the 316(a) samples as one of the more abundant of the intertidal invertebrates, it is present in the Diablo Cove vicinity and some populations of this species could be exposed to the thermal plume. The species was included in a 96-hour ET50 experiment that was conducted on several RIS because space was available in the tanks and only a minimal increase in effort was necessary to obtain this thermal tolerance information.

2.10.4.1 METHODS

A summary of the methods employed in the heat tolerance experiment on Acanthina spirata was presented in SECTION 2.4.2.1. A more detailed account of the experimental procedure used in 96-hour ET50 experiments is presented in APPENDIX B (Procedure 319). The protocol for this experiment is included in APPENDIX C (experiment 19c).

The adult specimens of A. spirata used in this experiment were collected on 20 June 1979 at the north side of Field's Cove, just north of Diablo Cove. The animals were held in the laboratory at ambient temperatures and were kept continuously submerged throughout their stay in the laboratory. During the holding period, the animals were carefully observed and those which appeared damaged or otherwise unhealthy were excluded from use in the experiment.

The animals were kept in the test and control tanks underneath a slip-fit coupling for 3 in. PVC pipe screened at one end with 1/8 in. mesh Vexar plastic. These cages were separated from other species run in the same experiment by being placed within a 12 x 18 in. compartment made of 1/8 in. Vexar in a 4 x 8 ft tank. Temperatures were controlled and recorded by an Autodata Nine computer. The temperature data were transcribed from paper tapes onto data sheets and summarized as cumulative means and standard deviations for each observation period.

Observations were made after 6 hours and subsequently every 12 hours for the remainder of the 96-hour test period. Experimental target temperatures were 19, 20.5, 22, 23.5, 25, 26.5 and 28C. An ambient control treatment was also maintained.

The criterion for death was the failure to retract into the shell when gently prodded.

The following species were run simultaneously in this 96-hour test: Cancer antennarius, Collisella digitalis, C. limatula, C. pelta, C. scabra, Hemigrapsus nudus, Ocenebra circumtexta, Pachygrapsus crassipes, Pugettia producta, P. richii, and Strongylocentrotus purpuratus.

2.10.4.2 RESULTS

Cumulative means and standard deviations of temperature data for each observation period are presented in TABLE 2.10.4-1.

No mortality was observed in any of the test or control tanks during or after the experiment.

TABLE 2.10.4-1

CUMULATIVE TEMPERATURE MEANS AND STANDARD DEVIATIONS (IN PARENTHESES) FOR ALL OBSERVATION PERIODS: ACANTHINA SPIRATA HEAT TOLERANCE

These data also apply to 96-hr ET50 tests on Collisella digitalis, C. limatula, C. pelta, C. scabra, Ocenebra circumtexta, Strongylocentrotus purpuratus and Tegula brunnea.

Target Temperature (C)	Observation Period (hours)									
	0	6	18	30	42	54	66	78	90	96
Ambient	13.1 (0.0)	13.3 (0.1)	12.7 (0.5)	12.6 (0.5)	12.4 (0.6)	12.3 (0.6)	12.3 (0.5)	12.5 (0.7)	12.6 (0.7)	12.6 (0.7)
19	18.7 (0.0)	19.0 (0.1)	18.9 (0.1)	19.0 (0.1)	19.0 (0.1)	19.0 (0.1)	19.0 (0.1)	19.0 (0.1)	19.0 (0.1)	19.0 (0.1)
20.5	20.6 (0.0)	20.6 (0.0)	20.6 (0.0)	20.6 (0.0)	20.6 (0.0)	20.6 (0.0)	20.6 (0.0)	20.6 (0.0)	20.6 (0.0)	20.6 (0.0)
22	21.8 (0.0)	22.1 (0.1)	22.0 (0.1)	22.0 (0.1)	22.0 (0.1)	22.0 (0.1)	22.0 (0.1)	22.0 (0.1)	22.0 (0.1)	22.1 (0.1)
23.5	23.3 (0.0)	23.5 (0.1)	23.5 (0.1)	23.5 (0.1)	23.5 (0.1)	23.5 (0.1)	23.5 (0.1)	23.5 (0.1)	23.5 (0.1)	23.5 (0.1)
25	24.8 (0.0)	24.9 (0.1)	24.9 (0.1)	24.9 (0.1)	24.9 (0.1)	24.9 (0.1)	24.9 (0.1)	24.9 (0.1)	24.9 (0.0)	24.9 (0.0)
26.5[a]	26.4 (0.0)	26.5 (0.0)	26.5 (0.0)	26.5 (0.0)	26.5 (0.0)	26.5 (0.0)	26.5 (0.0)	26.5 (0.0)	26.5 (0.0)	26.5 (0.0)
28	28.0 (0.0)	28.0 (0.0)	27.9 (0.0)	28.0 (0.0)	28.0 (0.0)	28.0 (0.1)	28.0 (0.1)	28.0 (0.1)	28.0 (0.1)	28.0 (0.1)

	0	12	24	36	48	60	66	96
26.5[b]	26.5 (0.0)	26.4 (0.0)	26.4 (0.0)	26.4 (0.0)	26.5 (0.1)	26.5 (0.0)	26.5 (0.0)	26.6 (0.1)

[a] Run 1

[b] Run 2

2.10.4.3 DISCUSSION

Based on the results of this experiment, seawater temperatures up to 28.0C (82.4F) for four days would be expected to cause no thermal mortality in populations of Acanthina spirata.

2.10.5 ACMAEA MITRA 96-HOUR MEDIAN EFFECTIVE TEMPERATURE (96 H ET50)

The limpet Acmaea mitra is not one of the RIS but it occurs in the Diablo Canyon area. Because space was available, it was incorporated into the 96-hour ET50 experiment along with the three RIS species. The results of this 96-hour test to determine the median effective temperature (ET50), i.e., the temperature at which 50 percent of the test population survived, are reported on in this section.

As far as is known, there are no published accounts of thermal effects studies on Acmaea mitra. The study objective was to determine the 96-hour ET50 value of this species as an index to its thermal tolerance. In addition, the time course of mortality was recorded for the 96-hour period. The purpose of this study was to provide some indication of the possible effects of the power plant heated discharge on Acmaea mitra populations in Diablo Cove.

2.10.5.1 METHODS

A summary description of the laboratory methods employed in the investigations into heat tolerance of Acmaea mitra was presented in SECTION 2.4.2. For a more detailed description of the laboratory methods see APPENDIX B (Procedure 319) and protocol see APPENDIX C (Experiment 19a). The animals used in this experiment were about 20-25 mm in diameter. They were collected subtidally just south of the power plant intake cove on 18 April 1979.

The animals were assumed to be acclimated to ambient temperatures. During the experiment the animals were maintained on "hanging habitats" which were suspended so that they were submerged but not touching any surrounding surface. The habitat consisted of a 6 in. diameter PVC pipe cut in half longitudinally and 6-8 in. wide. Temperature data were recorded hourly by an Autodata Nine computer, transcribed, and summarized as cumulative means and standard deviations at each observation period for each test tank.

Test animals were observed at 12-hour intervals during the 96-hour period at test temperatures of 15.1 C and ranging from 23.1 to 28.8 C. A control group of animals was maintained and observed at ambient temperatures.

Failure of an animal to hold onto a substrate constituted the criterion for "ecological death" (effective temperature, ET) during the experiment. Animals which met this criterion were returned to ambient temperatures to observe any recovery. Seven to seventeen animals were tested in each treatment group. Probit analysis of the mortality data was conducted in accordance with Standard Methods (1975).

2.10.5.2 RESULTS

Cumulative means and standard deviations of the temperature data for all test tanks are given in TABLE 2.4-6 (see SECTION 2.4.2).

No mortality was observed in the ambient and 15.1 C tanks. For the remaining tanks, the cumulative mortalities were plotted and are presented in FIGURE 2.10.5-1.

All "dead" animals were replaced into ambient seawater to determine if they would recover. At 162 hours, 4 of the 13 "dead" animals from the 23.9 C treatment had recovered, 2 of the 13 "dead" animals from the 24.9 treatment had recovered, 2 of the 11 "dead" animals from the 25.8 C treatment had recovered and 2 of the 12 "dead" animals from the 27.0 C treatment had recovered. Therefore, the criterion of ability to hold does not appear to indicate physiological death directly. The morphology of the animal and its shell is such that it would be very difficult for this animal to right itself and become reattached once it lost hold of the substrate, making it susceptible to predation in nature. With these points in mind, it is reasonable to assume that loss of ability to hold a surface by A. mitra constitutes "ecological death" in that few if any animals losing this ability would be expected to survive under natural conditions. The mortality data presented below represent the ecological deaths recorded at the 96-hour observation.

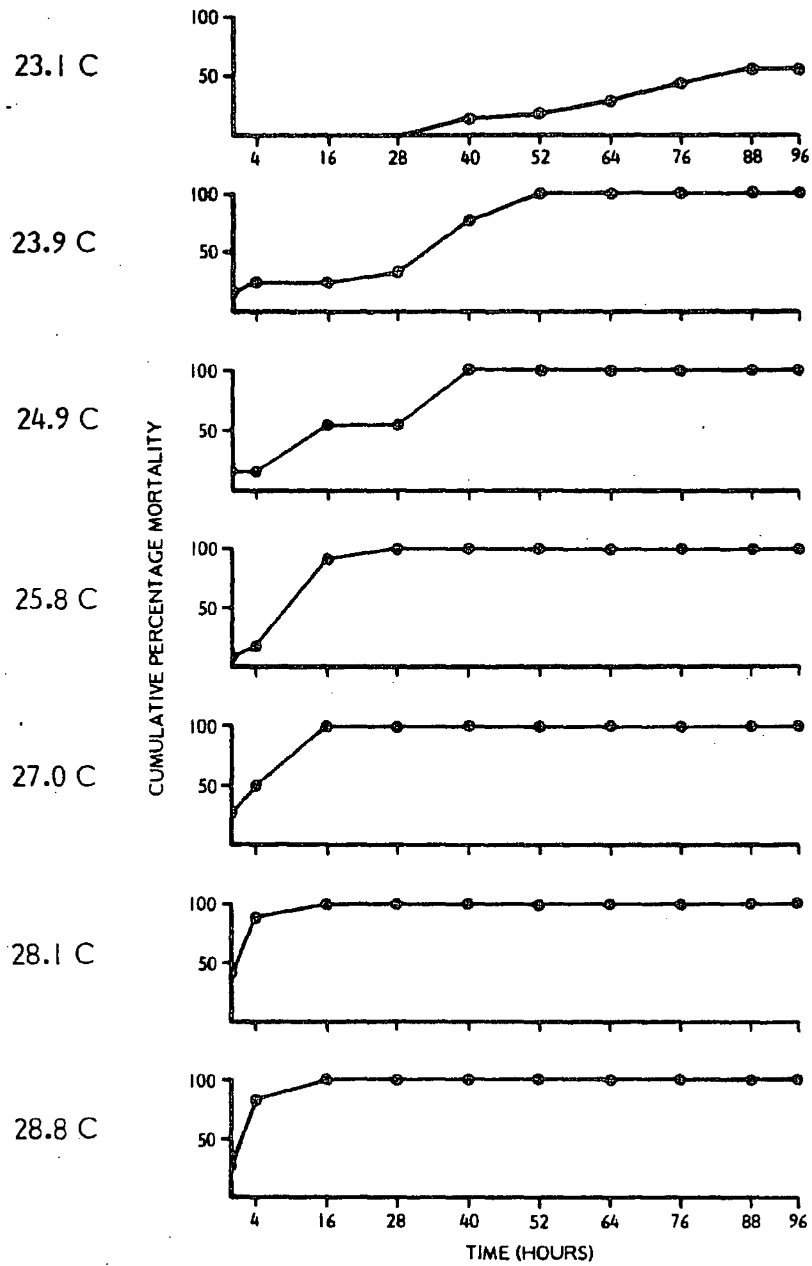

FIGURE 2.10.5-1

CUMULATIVE PERCENTAGE MORTALITY VERSUS TIME: ACMAEA MITRA HEAT TOLERANCE

The animals were acclimated to ambient temperatures.

All live animals at the end of the 96-hour test period were placed into ambient seawater to determine whether any "latent" mortality would occur. Only one latent mortality was observed in the animals from the 23.1 C treatment. This mortality was not included in the following analysis.

Probit regression lines were fitted by eye to determine the temperature at 50 percent mortality at each observation period. The 96-hour ET50 value was 23.1 C. Estimations of the temperatures at which 50 percent mortality occurred at each observation period were derived from the probability plots and used to generate a curve of time to 50 percent mortality versus temperature shown in FIGURE 2.10.5-2. The "incipient lethal temperature," which is indicated by a vertical alignment of points to the left, was defined in this experiment at approximately 23.1 C.

2.10.5.3 DISCUSSION

Using the criterion of lack of response to prodding to define death, seawater temperatures exceeding 23.1 C (73.6 F) for four days would be expected to cause one half of the population to die. Shorter exposures and lower temperatures would produce fewer mortalities.

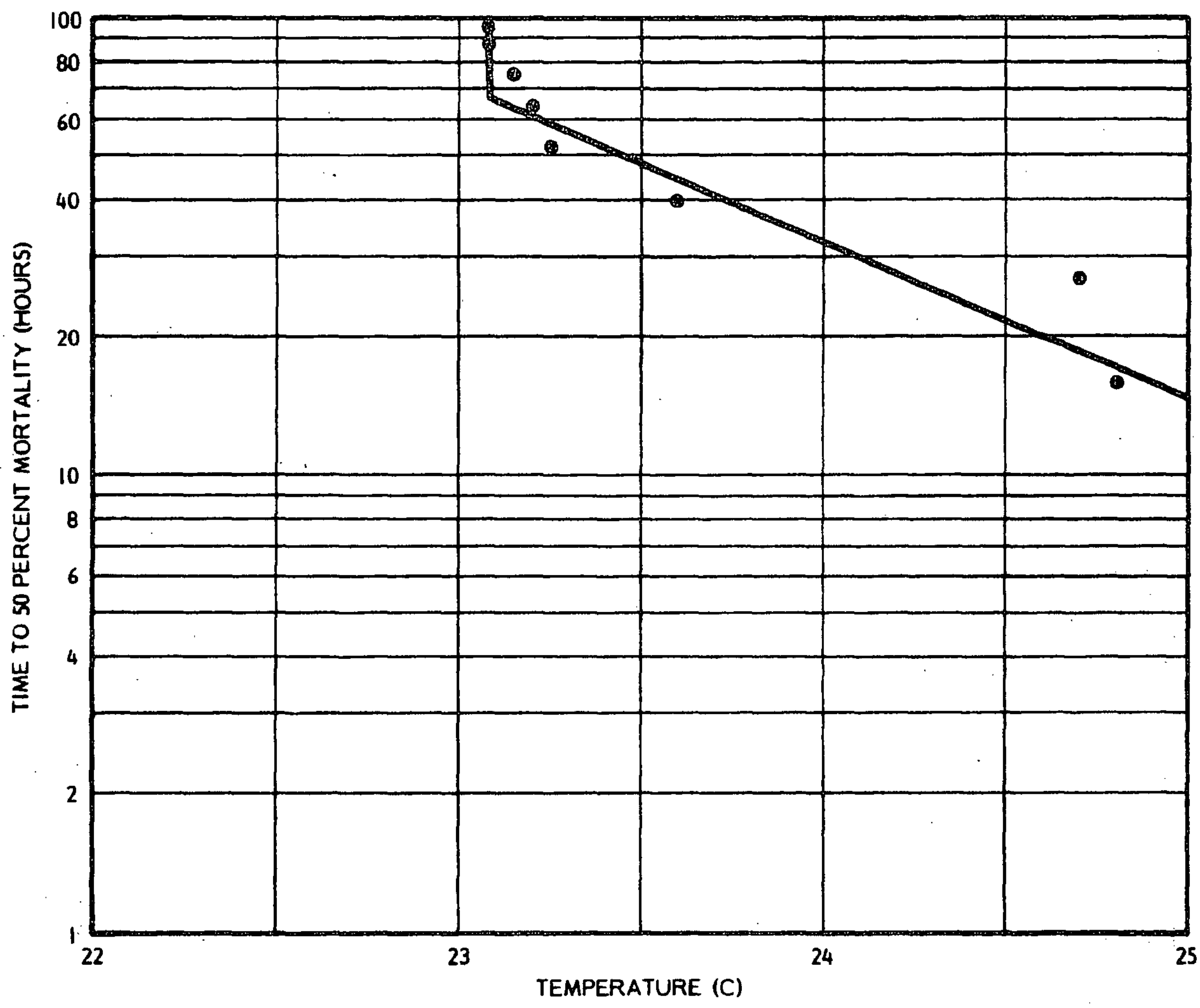

FIGURE 2.10.5-2

TIME TO 50 PERCENT MORTALITY VERSUS TEMPERATURE:
ACMAEA MITRA HEAT TOLERANCE

The animals were acclimated to ambient temperatures.

2.10.6 ASTRAEA GIBBEROSA 96-HOUR MEDIAN EFFECTIVE TEMPERATURE (96 H ET50)

The snail Astraea gibberosa is not one of the RIS but it occurs commonly in the Diablo Canyon area. Because space was available, it was incorporated into the 96-hour ET50 experiment along with the three RIS species. The results of this 96-hour test to determine the median effective temperature (ET50), i.e., the temperature at which 50 percent of the test population survived, are reported on in this section.

As far as is known, there are no published accounts of thermal effects studies on Astraea gibberosa. The study objective was to determine the 96-hour ET50 value of this species as an index to its thermal tolerance. In addition, the time course of mortality was recorded for the 96-hour period. The purpose of this study was to provide some indication of the possible effects of the power plant heated discharge on A. gibberosa populations in Diablo Cove.

2.10.6.1 METHODS

A summary description of the laboratory methods employed in the investigations into heat tolerance of Astraea gibberosa was presented in SECTION 2.4.2. For a more detailed description of the laboratory methods see APPENDIX B (Procedure 319) and protocol see APPENDIX C (Experiment 19a).

The animals used in this experiment were about 25-40 mm in diameter. They were collected subtidally just south of the power plant intake cove on 18 April 1979.

The animals were assumed to be acclimated to ambient temperatures. During the experiment the animals were maintained on "hanging habitats" which were suspended so that they were submerged but not touching any surrounding surface. The habitat consisted of a 6 in. diameter PVC pipe cut in half longitudinally and

6-8 in. wide. Temperature data were recorded hourly by an Autodata Nine computer, transcribed, and summarized as cumulative means and standard deviations at each observation period for each test tank.

Test animals were observed at 12-hour intervals during the 96-hour period at test temperatures of 15.1 C and ranging from 23.1 to 28.8 C. A control group of animals was maintained and observed at ambient temperatures.

Failure of an animal to retract into its shell after prodding constituted the criterion for "ecological death" (effective temperature, ET) during the experiment. Animals which met this criterion were returned to ambient temperatures to observe any recovery. Ten or eleven animals were tested in each treatment group. Probit analysis of the mortality data was conducted in accordance with Standard Methods (1975).

2.10.6.2 RESULTS

Cumulative means and standard deviations of the temperature data for all test tanks are given in TABLE 2.4-6 (see SECTION 2.4.2).

No mortality was observed in the ambient 15.1, 23.1, 23.9 and 24.9 C tanks. For the remaining tanks, the cumulative mortalities were plotted and are presented in FIGURE 2.10.6-1.

All "dead" animals were replaced into ambient seawater to determine if they would recover. At 162 hours, one of the "dead" animals from the 25.9 C tank had recovered. It may have succumbed just prior to the end of the 96-hour test period. This animal is counted as dead in the following analysis. All live animals at the end of the 96-hour test period were placed into ambient seawater to determine whether any "latent" mortality would occur. No latent mortalities were observed at 162 hours.

Probit regression lines were fitted by eye to determine the temperature at 50 percent mortality at each observation period. The 96-hour ET50 value was

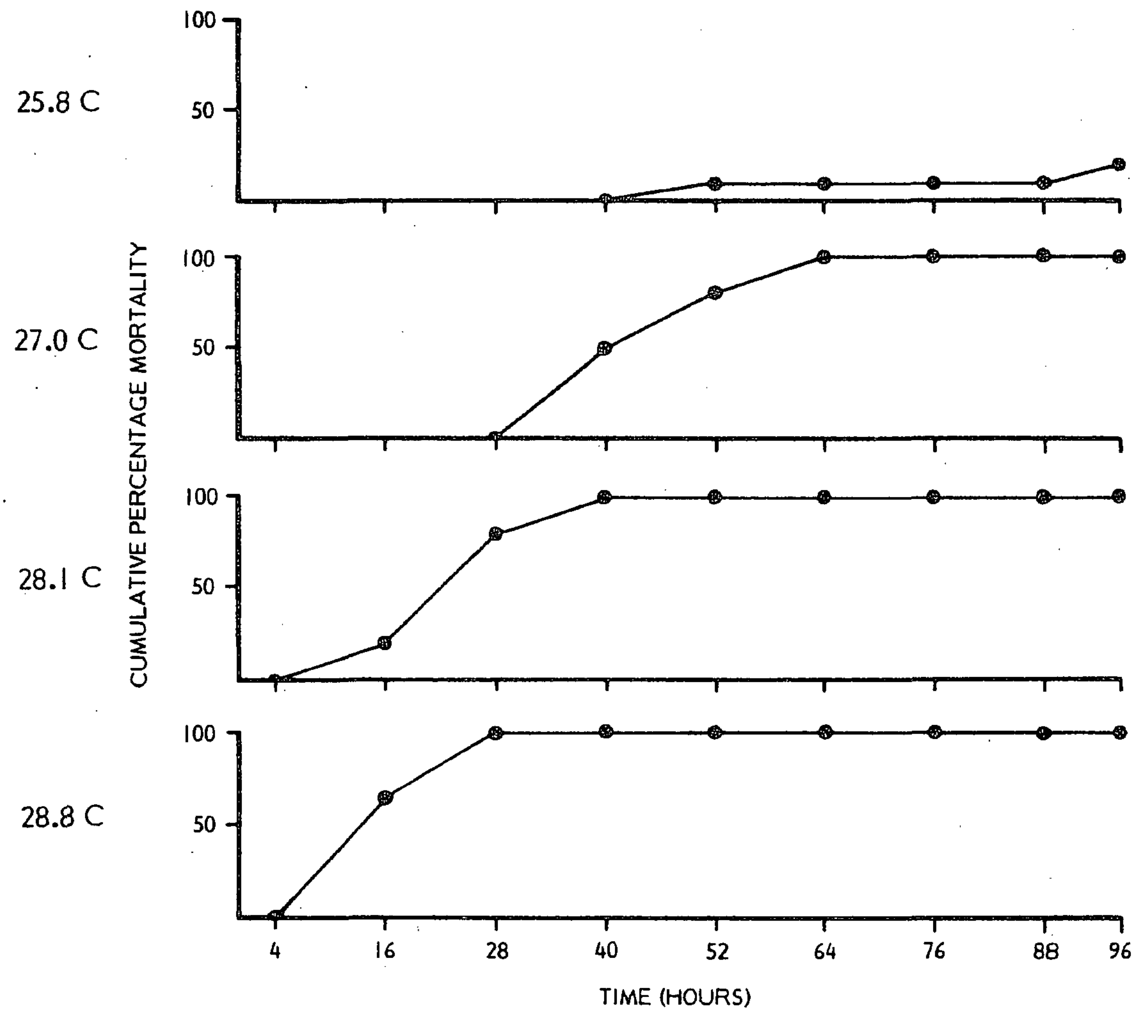

FIGURE 2.10.6-1

CUMULATIVE PERCENTAGE MORTALITY VERSUS TIME:
ASTRAEA GIBBEROSA HEAT TOLERANCE

The animals were acclimated to ambient temperatures.

26.0 C. Estimations of the temperatures at which 50 percent mortality occurred at each observation period were derived from the probability plots and used to generate a curve of time to 50 percent mortality versus temperature shown in FIGURE 2.10.6-2. The "incipient lethal temperature," which is indicated by a vertical alignment of points to the left, was defined in this experiment at approximately 26.0-26.1 C.

2.10.6.3 DISCUSSION

Using the criterion of lack of response to prodding to define death, seawater temperatures exceeding 26 C (78.8 F) for four days would be expected to cause one half of the population to die. Shorter exposures and lower temperatures would produce fewer mortalities and no thermal mortality would be expected at and below 24.9 C (76.8 F) for a four day exposure.

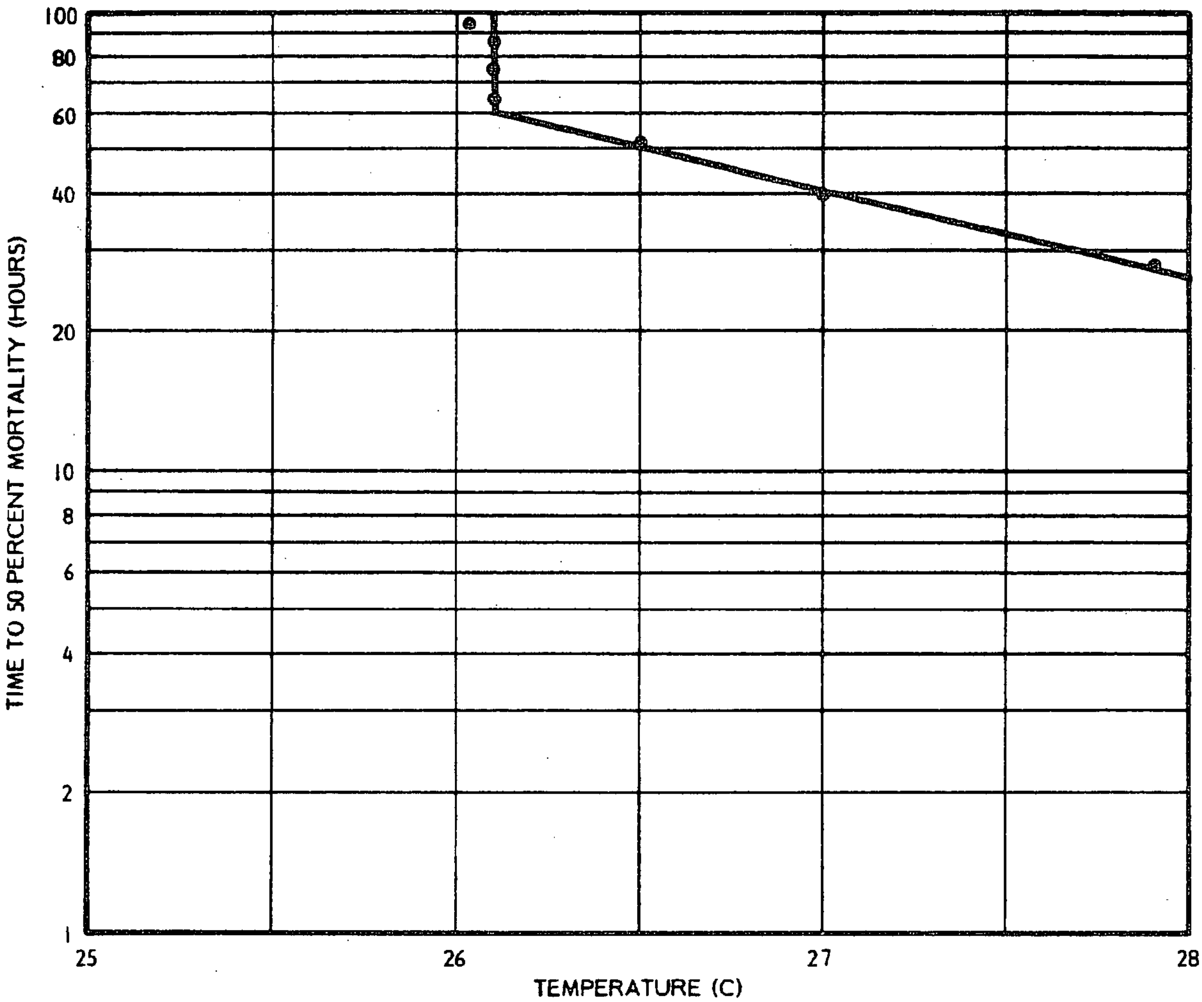

FIGURE 2.10.6-2

TIME TO 50 PERCENT MORTALITY VERSUS TEMPERATURE: ASTRAEA GIBBEROSA HEAT TOLERANCE

The animals were acclimated to ambient temperatures.

2.10.7 COLLISELLA DIGITALIS 96-HOUR MEDIAN EFFECTIVE TEMPERATURE (96 H ET50)

According to Morris et al. (1980), the ribbed limpet, Collisella digitalis, occurs commonly on vertical rock surfaces in the upper intertidal and splash zones. Its geographical distribution extends from the Aleutian Islands to southern Baja California, Mexico. It is one of the larger Pacific Coast limpets, with shell lengths of 15-30 mm. It feeds on microscopic films of algae in the high intertidal, often having a major effect on the algal crop there.

Although C. digitalis is not an RIS and has not been recorded in the 316(a) samples as one of the more abundant of the intertidal invertebrates, it is present in the Diablo Cove vicinity and some populations of this species could be exposed to the thermal plume. The species was included in a 96-hour ET50 experiment that was conducted on several RIS because space was available in the tanks and only a minimal increase in effort was necessary to obtain this thermal tolerance information.

2.10.7.1 METHODS

A summary of the methods employed in the heat tolerance experiment on Collisella digitalis was presented is SECTION 2.4.2. A more detailed account of the experimental procedure used in 96-hour ET50 experiments is presented in APPENDIX B (Procedure 319). The protocol for this experiment is included in APPENDIX C (experiment 19c).

The adult specimens of C. digitalis used in this experiment were collected on 11 July 1979 at "Seal Haulout," just south of Diablo Cove. The animals were held in the laboratory at ambient temperatures and were kept continuously submerged throughout their stay in the laboratory. During the holding period, the animals were carefully observed and those which appeared damaged or otherwise unhealthy were excluded from use in the experiment.

The animals were kept in the test and control tanks by means of "hanging habitats" which were constructed of 6-8 in. wide semicircles cut from 6 in. diameter PVC pipe. The habitats were suspended by stainless steel wire from supports stretching across the 4 x 8 ft tanks so that they were completely submerged but not touching any other objects in the tank. Temperatures were controlled and recorded by an Autodata Nine computer. The temperature data were transcribed from paper tapes onto data sheets and summarized as cumulative means and standard deviations for each observation period.

Observations were made after 6 hours and subsequently every 12 hours for the remainder of the 96-hour test period. Experimental target temperatures were 19, 20.5, 22, 23.5, 25, 26.5 and 28C. An ambient control treatment was also maintained.

The criterion for death was lack of ability of the animal to hold onto a surface.

The following species were run simultaneously in this 96-hour test: Acanthina spirata, Cancer antennarius, Collisella pelta, C. limatula, C. scabra, Hemigrapsus nudus, Ocenebra circumtexta, Pachygrapsus crassipes, Pugettia producta, P. richii, and Strongylocentrotus purpuratus.

2.10.7.2 RESULTS

Cumulative means and standard deviations of temperature data for each observation period are presented in TABLE 2.10.4-1

No mortality was observed in any of the test or control tanks.

2.10.7.3 DISCUSSION

Based on the results of this experiment, seawater temperatures up to 28.0C (82.4F) for four days would be expected to cause no thermal mortality in populations of Collisella digitalis from the Diablo Cove area.

2.10.8 COLLISELLA LIMATULA 96-HOUR MEDIAN EFFECTIVE TEMPERATURE (96 H ET50)

According to Morris et al. (1980), the file limpet, Collisella limatula, is abundant on semiprotected rocks in the middle to low intertidal zones. Its geographical distribution extends from Newport, Oregon to southern Baja California, Mexico. It is one of the larger Pacific Coast limpets, with shell lengths of 30-45 mm. It feeds on microscopic algae and on some of the larger crustose algae, both uncalcified and calcified.

Although C. limatula is not an RIS and has not been recorded in the 316(a) samples as one of the more abundant of the intertidal invertebrates, it is present in the Diablo Cove vicinity and some populations of this species could be exposed to the thermal plume. The species was included in a 96-hour ET50 experiment that was conducted on several RIS because space was available in the tanks and only a minimal increase in effort was necessary to obtain this thermal tolerance information.

2.10.8.1 METHODS

A summary of the methods employed in the heat tolerance experiment on Collisella limatula was presented in SECTION 2.4.2. A more detailed account of the experimental procedure used in 96-hour ET50 experiments is presented in APPENDIX B (Procedure 319). The protocol for this experiment is included in APPENDIX C (experiment 19c).

The adult specimens of C. limatula used in this experiment were collected on 13 July 1979 at the north side of "Field's Cove" just north of Diablo Cove. The animals were held in the laboratory at ambient temperatures and were kept continuously submerged throughout their stay in the laboratory. During the holding period, the animals were carefully observed and those which appeared damaged or otherwise unhealthy were excluded from use in the experiment.

The animals were kept in the test and control tanks by means of "hanging habitats" which were constructed of 6-8 in. wide semicircles cut from 6 in. diameter PVC pipe. The habitats were suspended by stainless steel wire from supports stretching across the 4 x 8 ft tanks so that they were completely submerged but not touching any other objects in the tank. Temperatures were controlled and recorded by an Autodata Nine computer. The temperature data were transcribed from paper tapes onto data sheets and summarized as cumulative means and standard deviations for each observation period.

Observations were made after 6 hours and subsequently every 12 hours for the remainder of the 96-hour test period. Experimental target temperatures were 19, 20.5, 22, 23.5, 25, 26.5 and 28C. An ambient control treatment was also maintained.

The criterion for death was lack of ability to hold onto a surface.

The following species were run simultaneously in this 96-hour test: Acanthina spirata, Cancer antennarius, Collisella digitalis, C. pelta, C. scabra, Hemigrapsus nudus, Ocenebra circumtexta, Pachygrapsus crassipes, Pugettia producta, P. richii, and Strongylocentrotus purpuratus.

2.10.8.2 RESULTS

Cumulative means and standard deviations of temperature data for each observation period are presented in TABLE 2.10.4-1.

No mortality was observed in any of the test or control tanks.

2.10.8.3 DISCUSSION

Based on the results of this experiment, seawater temperatures up to 28.0C (82.4F) for four days would be expected to cause no thermal mortality in populations of Collisella limatula from the Diablo Cove area.

2.10.9 COLLISELLA PELTA 96-HOUR MEDIAN EFFECTIVE TEMPERATURE (96 H ET50)

According to Morris et al. (1980), the shield limpet, Collisella pelta, occurs commonly on rocky reefs in the middle to low intertidal zones. Its geographical distribution extends from the Aleutian Islands to Bahia del Rosario, Baja California, Mexico. It is one of the larger Pacific Coast limpets, with shell lengths up to 40 mm. It feeds on microscopic and macroscopic algae, especially the common erect algae Endocladia, Rhodoglossum, Iridaea, Pelvetia, Egregia and Postelsia.

Although C. pelta is not an RIS and has not been recorded in the 316(a) samples as one of the more abundant of the intertidal invertebrates, it is present in the Diablo Cove vicinity and some populations of this species could be exposed to the thermal plume. The species was included in a 96-hour ET50 experiment that was conducted on several RIS because space was available in the tanks and only a minimal increase in effort was necessary to obtain this thermal tolerance information.

2.10.9.1 METHODS

A summary of the methods employed in the heat tolerance experiment on Collisella pelta was presented in SECTION 2.4.2. A more detailed account of the experimental procedure used in 96-hour ET50 experiments is presented in APPENDIX B (Procedure 319). The protocol for this experiment is included in APPENDIX C (experiment 19c).

The adult specimens of C. pelta used in this experiment were collected on 11 July 1979 at "Seal Haulout," just south of Diablo Cove. The animals were held in the laboratory at ambient temperatures and were kept continuously submerged throughout their stay in the laboratory. During the holding period, the animals were carefully observed and those which appeared damaged or otherwise unhealthy were excluded from use in the experiment.

The animals were kept in the test and control tanks by means of "hanging habitats" which were constructed of 6-8 in. wide semicircles cut from 6 in. diameter PVC pipe. The habitats were suspended by stainless steel wire from supports stretching across the 4 x 8 ft tanks so that they were completely submerged but not touching any other objects in the tank. Temperatures were controlled and recorded by an Autodata Nine computer. The temperature data were transcribed from paper tapes onto data sheets and summarized as cumulative means and standard deviations for each observation period.

Observations were made after 6 hours and subsequently every 12 hours for the remainder of the 96-hour test period. Experimental target temperatures were 19, 20.5, 22, 23.5, 25, 26.5 and 28C. An ambient control treatment was also maintained.

The criterion for death was lack of ability to hold onto a surface. Animals that were presumed dead were returned to an ambient temperature tank to assess possible recovery. Mortality data were analyzed with probit analysis in accordance with Standard Methods (1976).

The following species were run simultaneously in this 96-hour test: Acanthina spirata, Cancer antennarius, Collisella digitalis, C. limatula, C. scabra, Hemigrapsus nudus, Ocenebra circumtexta, Pachygrapsus crassipes, Pugettia producta, P. richii, and Strongylocentrotus purpuratus.

2.10.9.2 RESULTS

Cumulative means and standard deviations of temperature data for each observation period are presented in TABLE 2.10.4-1.

Some mortality occurred over the entire range of temperatures in this experiment. One animal each succumbed in the ambient tank at 90 hours, the 20.5C tank at 42 hours, and the 22C tank at 90 hours. It is here assumed that none of these mortalities are directly attributable to thermal stress. Most likely, they resulted from stress caused by collection. The mortalities that occurred in the

25.0, 26.5 and 28.0C tanks are assumed to be attributable to thermal stress. Plots of the mortality percentage versus time for each of these tanks are presented in FIGURE 2.10.9-1. None of the presumed dead animals recovered after being placed in the ambient temperature recovery tank.

Probit regression lines were fitted by eye to the mortality data to determine the temperature which would cause 50 percent mortality at each observation period (see FIGURE 2.10.9-2). The 96-hour ET50 value was determined to be approximately 26.2C. The estimators of the ET50 value thus calculated for each observation period were used to generate a curve of time to 50 percent mortality versus temperature, which is presented in FIGURE 2.10.9-3. The "incipient lethal" temperature, indicated by the vertical alignment of points to the left of the curve, was not defined by the experiment.

2.10.9.3 DISCUSSION

Based on the results of this experiment, seawater temperatures in excess of 26.2C (79.2F) for four days would be expected to cause at least 50 percent mortality in a population of Collisella pelta. Little or no thermal mortality would be expected for exposures to temperatures below 25.0C (77.0F) for the same period of time. Exposure to 28.0C (82.4F) for 66 hours or more would be expected to cause 100 percent mortality in populations of C. pelta. However, it should be noted that C. pelta occurs in the intertidal region, alternately submerged and exposed to the air, whereas in this experiment, thermal exposure was induced by continual submergence. The environmental regime to which C. pelta is exposed in nature could have an ameliorative effect on thermal stress responses that were obtained in this experiment.

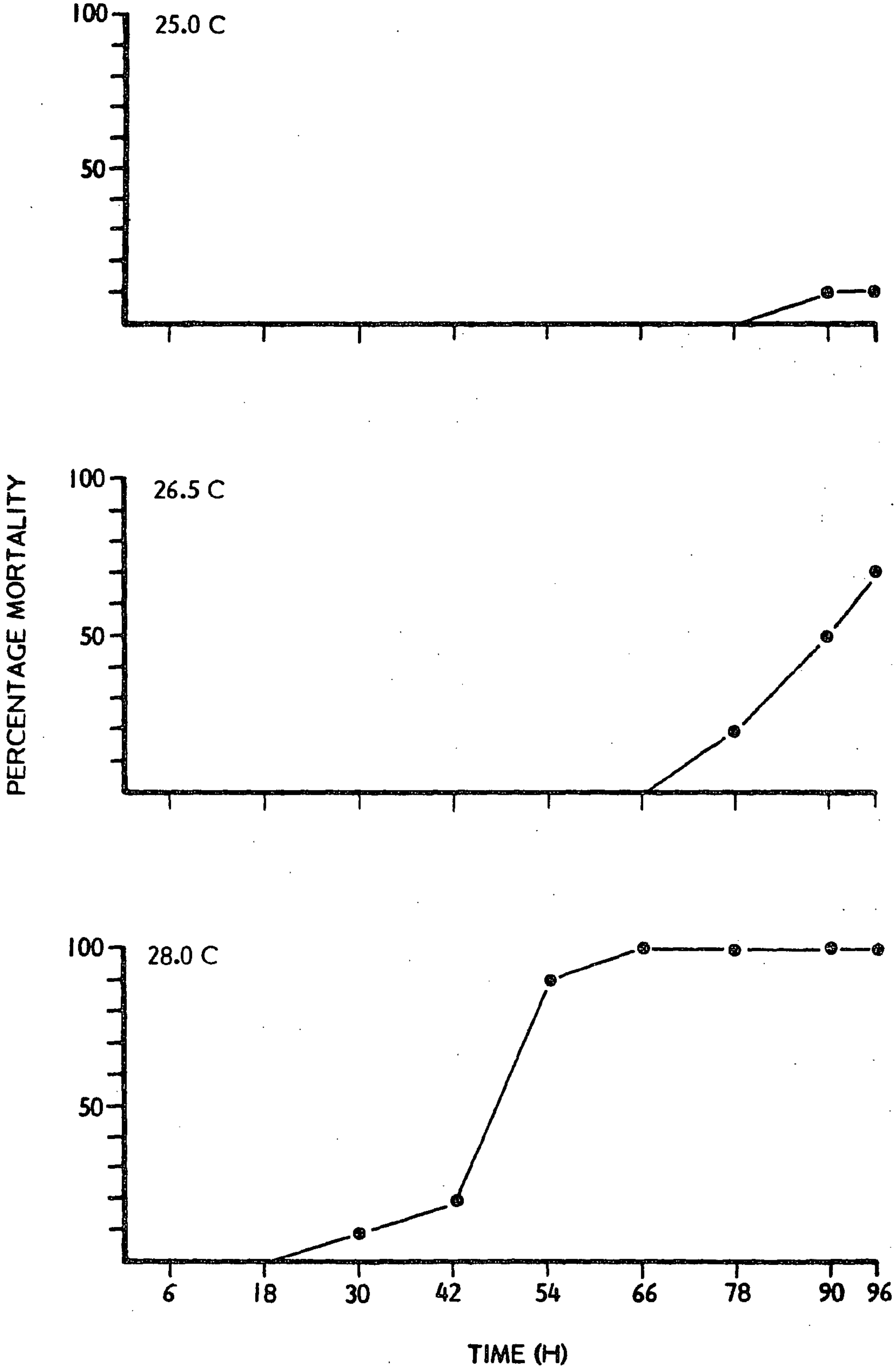

FIGURE 2.10.9-1

CUMULATIVE MORTALITY VERSUS TIME:
COLLISELLA PELTA HEAT TOLERANCE

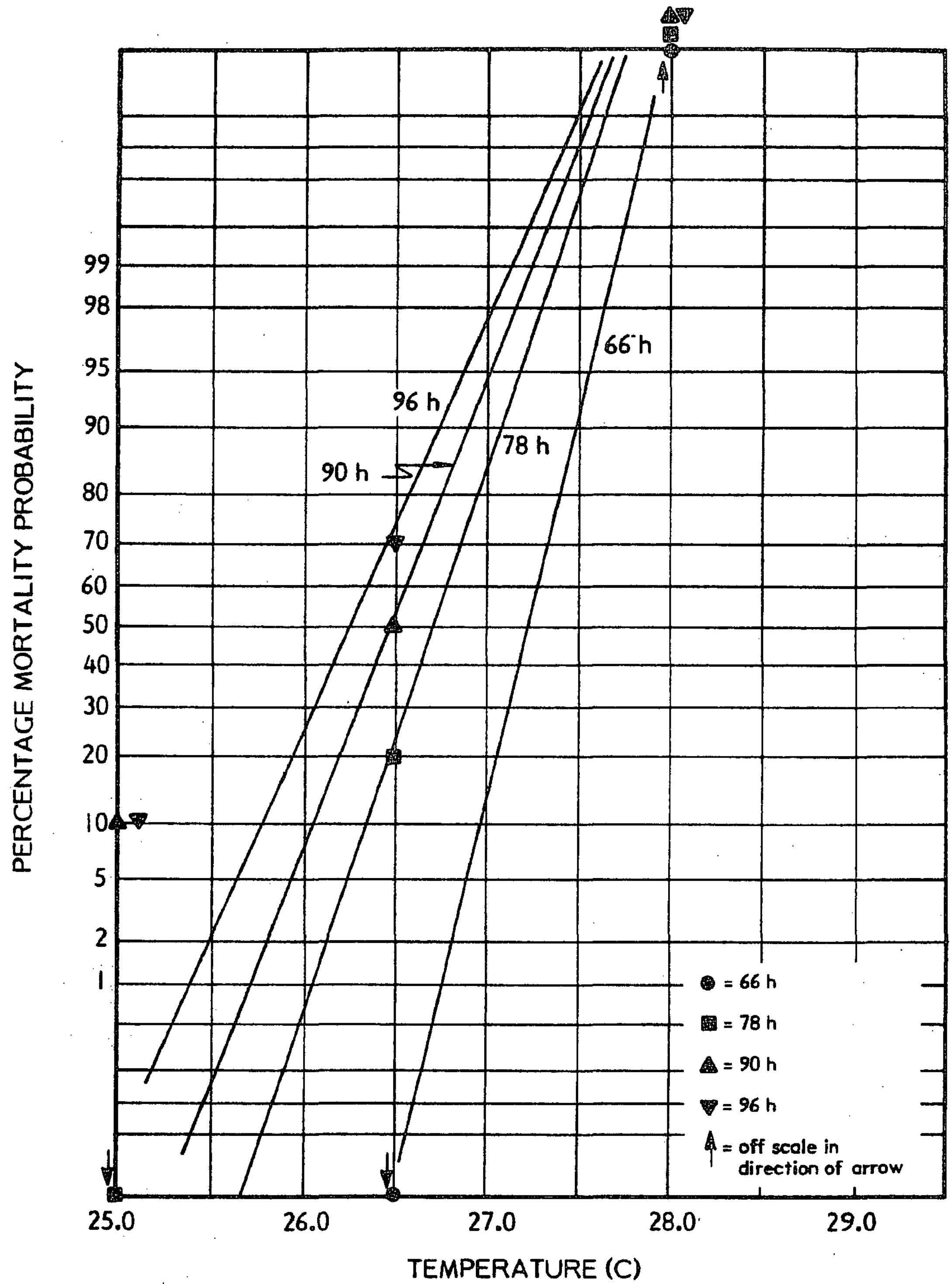

FIGURE 2.10.9-2

PERCENTAGE MORTALITY PROBABILITY VERSUS TEMPERATURE: COLLISELLA PELTA HEAT TOLERANCE

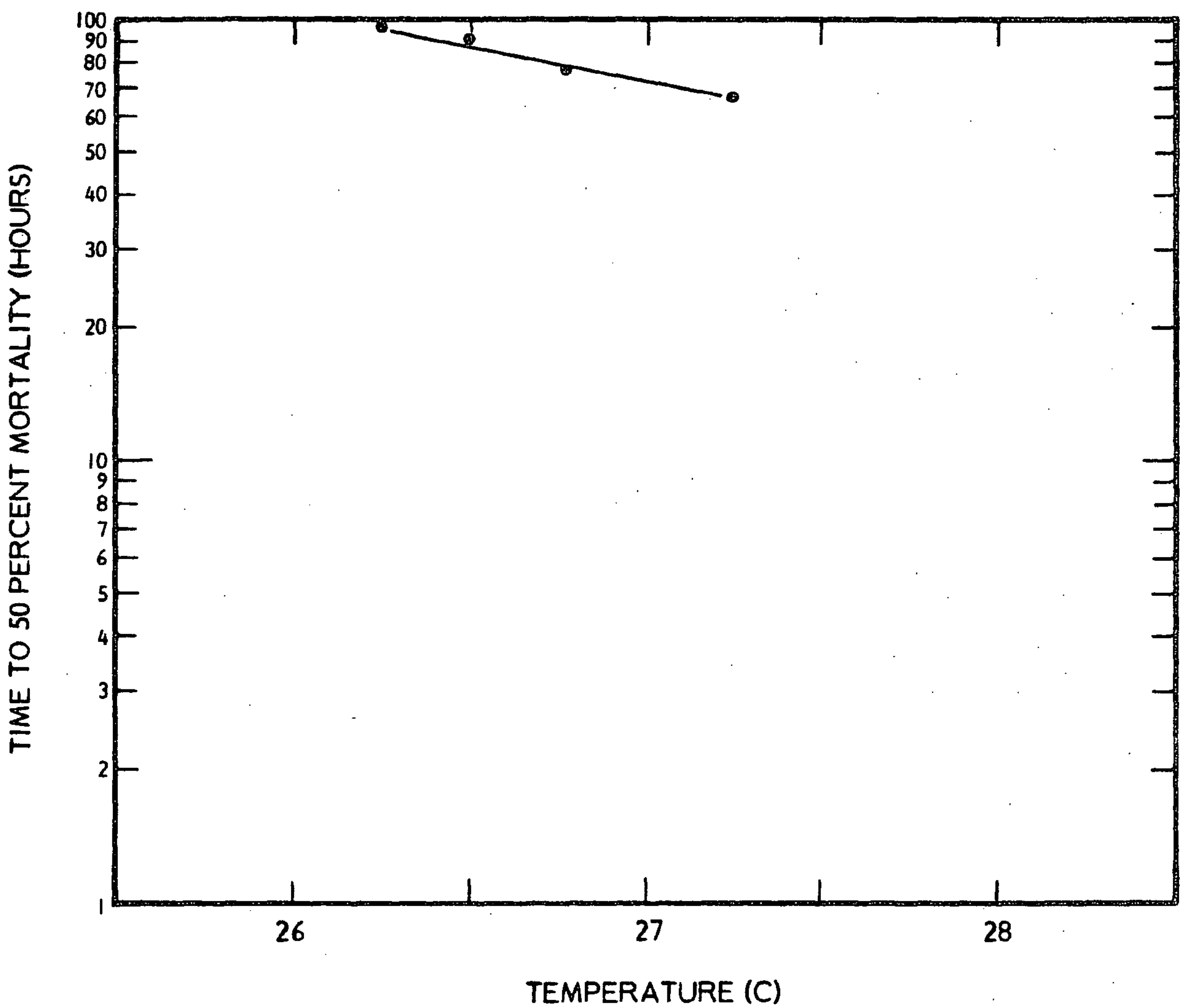

FIGURE 2.10.9-3

TIME TO 50 PERCENT MORTALITY VERSUS TEMPERATURE: *COLLISELLA PELTA* HEAT TOLERANCE

2.10.10 COLLISELLA SCABRA 96-HOUR MEDIAN EFFECTIVE TEMPERATURE (96 H ET50)

According to Morris et al. (1980), the rough limpet, Collisella scabra, occurs commonly on horizontal or gently sloping rock surfaces in the upper intertidal and splash zones. Its geographical distribution extends from Cape Arago, Oregon to southern Baja California, Mexico. It is a moderately large Pacific Coast limpet with shell lengths up to 30 mm. It feeds on microscopic films of algae in the high intertidal, often having a major effect on the algal crop there. The lower populations of C. scabra are subject to predation by the RIS sea star, Pisaster ochraceus.

Although C. scabra is not an RIS and has not been recorded in the 316(a) samples as one of the more abundant of the intertidal invertebrates, it is present in the Diablo Cove vicinity and some populations of this species could be exposed to the thermal plume. The species was included in a 96-hour ET50 experiment that was conducted on several RIS because space was available in the tanks and only a minimal increase in effort was necessary to obtain this thermal tolerance information.

2.10.10.1 METHODS

A summary of the methods employed in the heat tolerance experiment on Collisella scabra was presented in SECTION 2.4.2. A more detailed account of the experimental procedure used in 96-hour ET50 experiments is presented in APPENDIX B (Procedure 319). The protocol for this experiment is included in APPENDIX C (experiment 19c).

The adult specimens of C. scabra used in this experiment were collected on 11 July 1979 at "Seal Haulout," just south of Diablo Cove. The animals were held in the laboratory at ambient temperatures and were kept continuously submerged throughout their stay in the laboratory. During the holding period, the animals were carefully observed and those which appeared damaged or otherwise unhealthy were excluded from use in the experiment.

The animals were kept in the test and control tanks by means of "hanging habitats" which were constructed of 6-8 in. wide semicircles cut from 6 in. diameter PVC pipe. The habitats were suspended by stainless steel wire from supports stretching across the 4 x 8 ft tanks so that they were completely submerged but not touching any other objects in the tank. Temperatures were controlled and recorded by an Autodata Nine computer. The temperature data were transcribed from paper tapes onto data sheets and summarized as cumulative means and standard deviations for each observation period.

Observations were made after 6 hours and subsequently every 12 hours for the remainder of the 96-hour test period. Experimental target temperatures were 19, 20.5, 22, 23.5, 25, 26.5 and 28C. An ambient control treatment was also maintained.

The criterion for death was lack of ability to hold onto a surface. Animals that were presumed dead were returned to an ambient temperature tank to assess possible recovery.

The following species were run simultaneously in this 96-hour test: Acanthina spirata, Cancer antennarius, Collisella digitalis, C. limatula, C. pelta, Hemigrapsus nudus, Ocenebra circumtexta, Pachygrapsus crassipes, Pugettia producta, P. richii, and Strongylocentrotus purpuratus.

2.10.10.2 RESULTS

Cumulative means and standard deviations of temperature data for each observation period are presented in TABLE 2.10.4-1

During the experiment only one mortality was observed, in the 26.5C tank at 78 hours. Because no mortalities were observed in the 28.0C tank, it is here presumed that the single mortality resulted not from thermal stress but most likely from damage inflicted during collection. The presumed dead animal did not recover after being placed in the ambient temperature recovery tank.

2.10.10.3 DISCUSSION

Based on the results of this experiment, seawater temperatures up to 28.0C (82.4 F) for four days would be expected to cause no thermal mortality in populations of <u>Collisella</u> <u>scabra</u>.

2.10.11 HEMIGRAPSUS NUDUS 96-HOUR MEDIAN EFFECTIVE TEMPERATURE (96 H ET50)

The purple beach crab, Hemigrapsus nudus, is also an abundant invertebrate in the intertidal and shallow subtidal regions of the Diablo Canyon study area. Since no information has been published to date on this species' heat tolerance, an experiment was conducted to determine the 96-hour ET50 value. This value will serve as an index to the species thermal tolerance.

2.10.11.1 METHODS

A summary of the methods employed in the heat tolerance experiment on this species was presented in SECTION 2.4.2. A more detailed description of the experimental procedure utilized in 96-hour ET50 experiments on invertebrates is presented in APPENDIX B (Procedure 319). The protocol for this experiment is included in APPENDIX C (experiment 19c).

Specimens tested included both juveniles and adults that were collected by hand at low tide. Specimens were acclimated to laboratory conditions at ambient temperature prior to testing. During the experiment crabs were kept completely submerged in test tanks which were subdivided to accommodate several species per test. Temperature was controlled and recorded by an Autodata Nine computer. Temperature data were transcribed and summarized as cumulative means and standard deviations for each observation period.

Observations were made after six hours and subsequently every 12 hours for the 96-hour test period. Experimental target temperatures were 19, 20.5, 22, 23.5, 25, 26.5, and 28 C. An ambient control (mean of 12.5 C) was also run. Test populations consisted of ten animals per treatment.

The criterion for death was the loss of ability to walk when gently prodded. Animals that were presumed dead were returned to an ambient temperature tank to assess possible recovery.

2.10.11.2 RESULTS

TABLE 2.4-6 presents cumulative temperature means and standard deviations for each observation period. The time course of mortality is presented in FIGURE 2.10.11-1. After 96 hours of exposure, 10 percent of the test groups at 19, 23.5 and 25 C had died, while 20 percent of the animals in the 22 and 25 C tanks died. No mortalities occurred below 22 C, and the highest temperature tested, 28 C, also resulted in no mortalities after 96 hours. None of the presumed dead animals recovered after being placed in the ambient temperature recovery tank. The 50 percent mortality level was not reached at any temperature.

These results, while inconclusive in terms of defining the 50 percent mortality level at 96-hour exposure, do indicate that this species is highly tolerant of elevated temperatures.

2.10.11.3 DISCUSSION

The results of this experiment indicate that the purple beach crab, Hemigrapsus nudus, is able to tolerate temperatures up to 28 C (82.4 F) with only incidental mortalities.

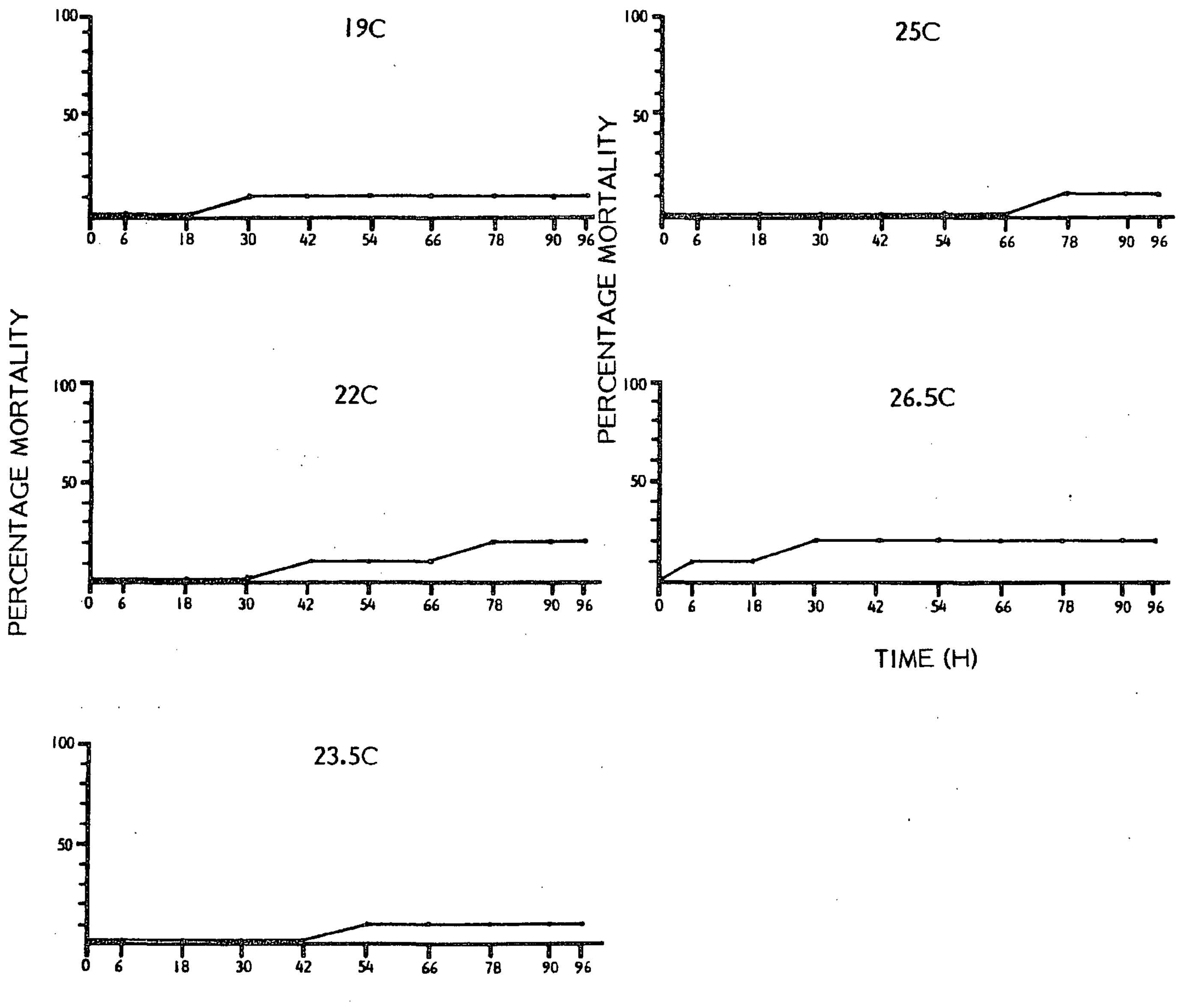

FIGURE 2.10.11-1

CUMULATIVE MORTALITY VERSUS TIME:
HEMIGRAPSUS NUDUS HEAT TOLERANCE

2.10.12 LEPTASTERIAS HEXACTIS 96-HOUR MEDIAN EFFECTIVE TEMPERATURE (96 H ET50)

According to Morris et al. (1980), Leptasterias hexactis occurs commonly on rocky shores in the middle intertidal zone. The species ranges into the subtidal to 208 m (684 ft) and geographically from Alaska to Santa Catalina Island of the Channel Islands off southern California (Gotshall and Laurent 1979, Morris et al. 1980). L. hexactis is a carnivore that feeds on many different prey (a "food generalist") and is often in direct competition for such prey with the RIS Pisaster ochraceus (Morris et al. 1980).

Although L. hexactis is not an RIS and has not been recorded in the 316(a) samples as one of the more abundant of the intertidal invertebrates, it is present in the Diablo Cove vicinity and some populations of this species could be exposed to the thermal plume. The species was included in a 96-hour ET50 experiment that was conducted on several RIS because space was available in the tanks and only a minimal increase in effort was necessary to obtain this thermal tolerance information.

2.10.12.1 METHODS

A summary of the methods employed in the heat tolerance experiment on Leptasterias hexactis was presented in SECTION 2.4.2. A more detailed account of the experimental procedure used in 96-hour ET50 experiments is presented in APPENDIX B (Procedure 319). The protocol for this experiment is included in APPENDIX C (experiment 19b).

The adult specimens of L. hexactis used in this experiment were collected on 11 July 1979 at "Seal Haulout," just south of Diablo Cove. The animals were held in the laboratory at ambient temperatures and were kept continuously submerged throughout their stay in the laboratory. During the holding period, the animals were carefully observed and those which appeared damaged or otherwise unhealthy were excluded from use in the experiment.

The animals were kept in the test and control tanks in a 5-gal white plastic bucket on concrete building blocks. The bucket was supplied with a standpipe to maintain the water level just below the rim, and water and air supply hoses were placed in the bucket. The air line was connected to an airstone. The animals were continuously submerged during the course of the experiment. Temperatures were controlled and recorded by an Autodata Nine computer. The temperature data were transcribed from paper tapes onto data sheets and summarized as cumulative means and standard deviations for each observation period.

Observations were made after 8 hours and subsequently every 12 hours for the remainder of the 96-hour test period. Experimental target temperatures were 22, 23, 24, 25, 26, 27 and 28C. An ambient control treatment was also maintained. The number of animals tested was six each for the ambient and 26C, and seven each for the remainder of the test temperature treatments.

The criteria for death were the lack of ability to hold onto a surface and/or the absence of tube feet movement. Animals that were presumed dead were returned to an ambient temperature tank to assess possible recovery. Animals remaining alive at the end of the 96-hour test period were returned to ambient temperature. After about 72 hours (167 hours after the start of the 96-hour test) the animals were observed to determine if any "latent" mortality occurred. Mortality data were analyzed with probit analysis in accordance with Standard Methods (1976).

The following species were run simultaneously in this 96-hour test: Pagurus granosimanus, P. samuelis, Patiria miniata, Petrolisthes cinctipes, Pisaster brevispinus, P. giganteus and P. ochraceus.

2.10.12.2 RESULTS

Cumulative means and standard deviations of temperature data for each observation period are presented in TABLE 2.10.12-1.

TABLE 2.10.12-1

CUMULATIVE TEMPERATURE MEANS AND STANDARD DEVIATIONS (IN PARENTHESES) FOR ALL OBSERVATION PERIODS: *LEPTASTERIAS HEXACTIS* HEAT TOLERANCE EXPERIMENT

These data also apply to 96-hour ET50 tests on *Patiria miniata*, *Pisaster brevispinus*, *P. giganteus*, *P. ochraceus* and *Tegula brunnea*.

Target Temperature (C)	Observation Period (hours)									
	0	8	20	32	44	56	68	80	92	96
Ambient	13.6 (0.0)	14.4 (0.3)	14.2 (0.2)	14.4 (0.5)	14.4 (0.5)	14.3 (0.5)	14.4 (0.5)	14.5 (0.6)	14.5 (0.6)	14.5 (0.6)
22	22.2 (0.0)	22.2 (0.0)	22.2 (0.0)	22.2 (0.0)	22.2 (0.0)	22.2 (0.0)	22.2 (0.0)	22.2 (0.0)	22.2 (0.0)	22.2 (0.0)
23	23.1 (0.0)	23.1 (0.0)	23.1 (0.0)	23.1 (0.0)	23.1 (0.0)	23.1 (0.0)	23.1 (0.0)	23.1 (0.0)	23.1 (0.0)	23.1 (0.0)
24	23.9 (0.0)	24.0 (0.0)	24.0 (0.0)	23.9 (0.0)	24.0 (0.0)	23.9 (0.0)	23.9 (0.0)	24.0 (0.0)	24.0 (0.0)	24.0 (0.0)
25	25.0 (0.0)	25.0 (0.0)	25.0 (0.0)	25.0 (0.0)	25.0 (0.0)	25.0 (0.0)	25.0 (0.0)	25.0 (0.0)	25.0 (0.0)	25.0 (0.0)
26	25.4 (0.0)	25.7 (0.0)	25.7 (0.1)	25.7 (0.1)	25.7 (0.1)	25.7 (0.1)	25.7 (0.1)	25.7 (0.1)	25.7 (0.1)	25.7 (0.1)
27	27.0 (0.0)	27.0 (0.0)	27.1 (0.0)	27.0 (0.0)	27.0 (0.0)	27.0 (0.0)	27.0 (0.0)	27.0 (0.0)	27.0 (0.0)	27.0 (0.0)
28	28.0 (0.0)	27.9 (0.0)	28.0 (0.1)	28.0 (0.1)	28.0 (0.0)	28.0 (0.0)	28.0 (0.0)	28.0 (0.1)	27.9 (0.2)	27.9 (0.2)

Mortality occurred in the 23-28C tanks. Plots of the percentage mortality versus time for each of these treatments are presented in FIGURE 2.10.12-1. Of the presumed dead animals, one, from the 23C tank, recovered after being placed in the ambient temperature recovery tank. Because it is not known which of the animals recorded as presumed dead in the test tank recovered, the mortality data were not altered to reflect this fact. All other presumed dead animals did not recover. None of the animals that were alive at the end of the 96-hour period showed "latent" mortality.

Probit regression lines were fitted by eye to the mortality data to determine the temperature which would cause 50 percent mortality at each observation period (FIGURE 2.10.12-2). The 96-hour ET50 value was determined to be approximately 23C. The estimators of the ET50 value thus calculated for each observation period were used to generate a curve of time to 50 percent mortality versus temperature, which is presented in FIGURE 2.10.12-3. The "incipient lethal" temperature, indicated by the vertical alignment of points to the left of the curve, was defined by the experiment to be 23.0C, which is equivalent to the 96-hour ET50 value.

2.10.12.3 DISCUSSION

Based on the results of this experiment, seawater temperatures in excess of 23.0C (73.4F) for four days would be expected to cause 50 percent mortality in a population of <u>Leptasterias hexactis</u>. Little or no thermal mortality would be expected for exposures to temperatures at or below 22.0C (71.6F) for the same period of time. Exposure to 24C (75F) for 68 hours, 25C (77F) for 44 hours, 26C (79F) for 20 hours, 27C (81F) for 20 hours and 28C (82F) for 8 hours would be expected to cause 100 percent mortality in a population of <u>L. hexactis</u>. However, it should be noted that <u>L. hexactis</u> occurs most abundantly in the intertidal region, alternately submerged and exposed to the air, whereas in this experiment thermal exposure occurred during continual submergence. The environmental regime to which <u>L. hexactis</u> is exposed in nature could have an ameliorative effect on the thermal stress responses obtained in this experiment.

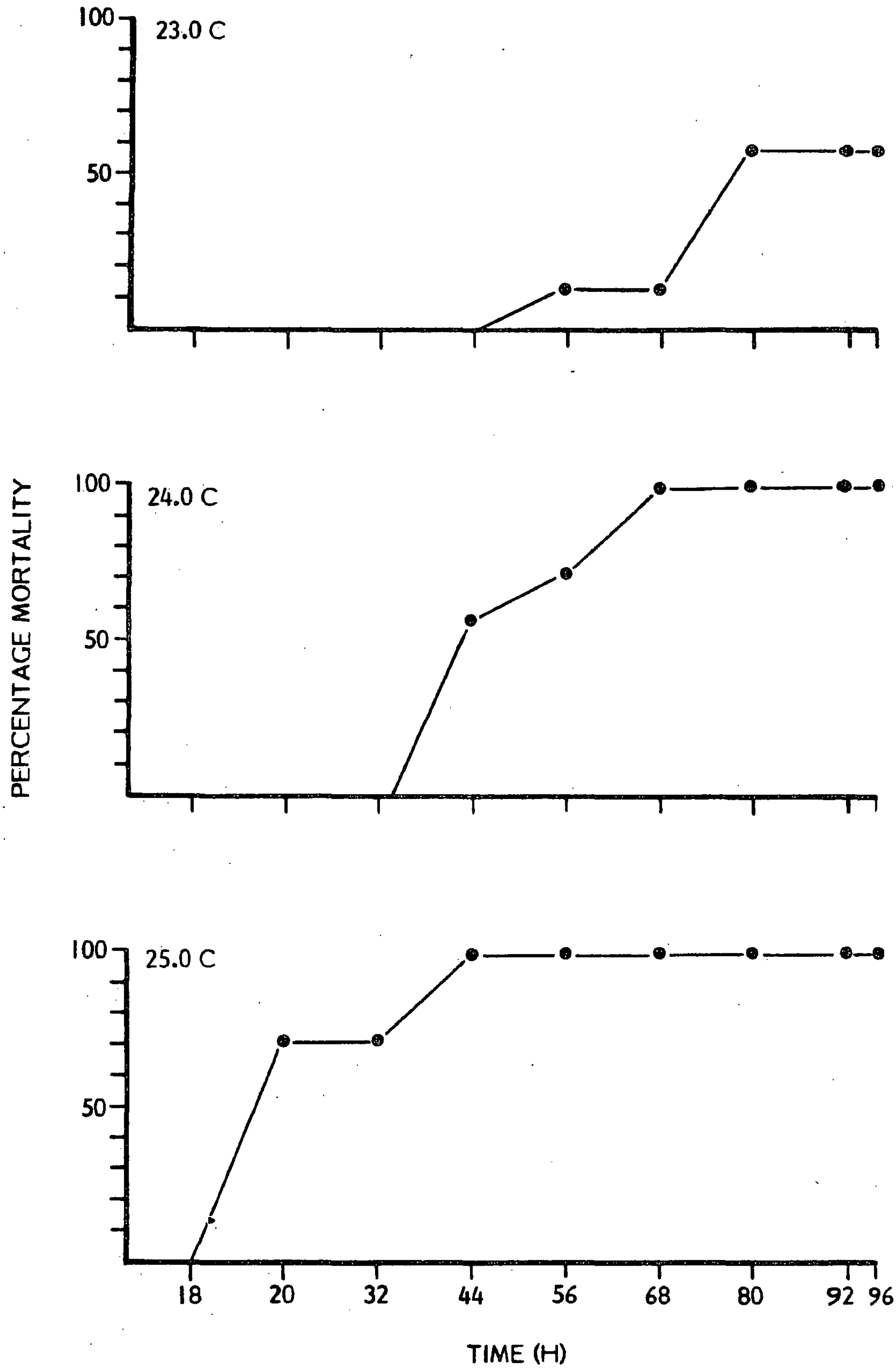

FIGURE 2.10.12-1

CUMULATIVE MORTALITY VERSUS TIME:
LEPTASTERIAS HEXACTIS HEAT TOLERANCE

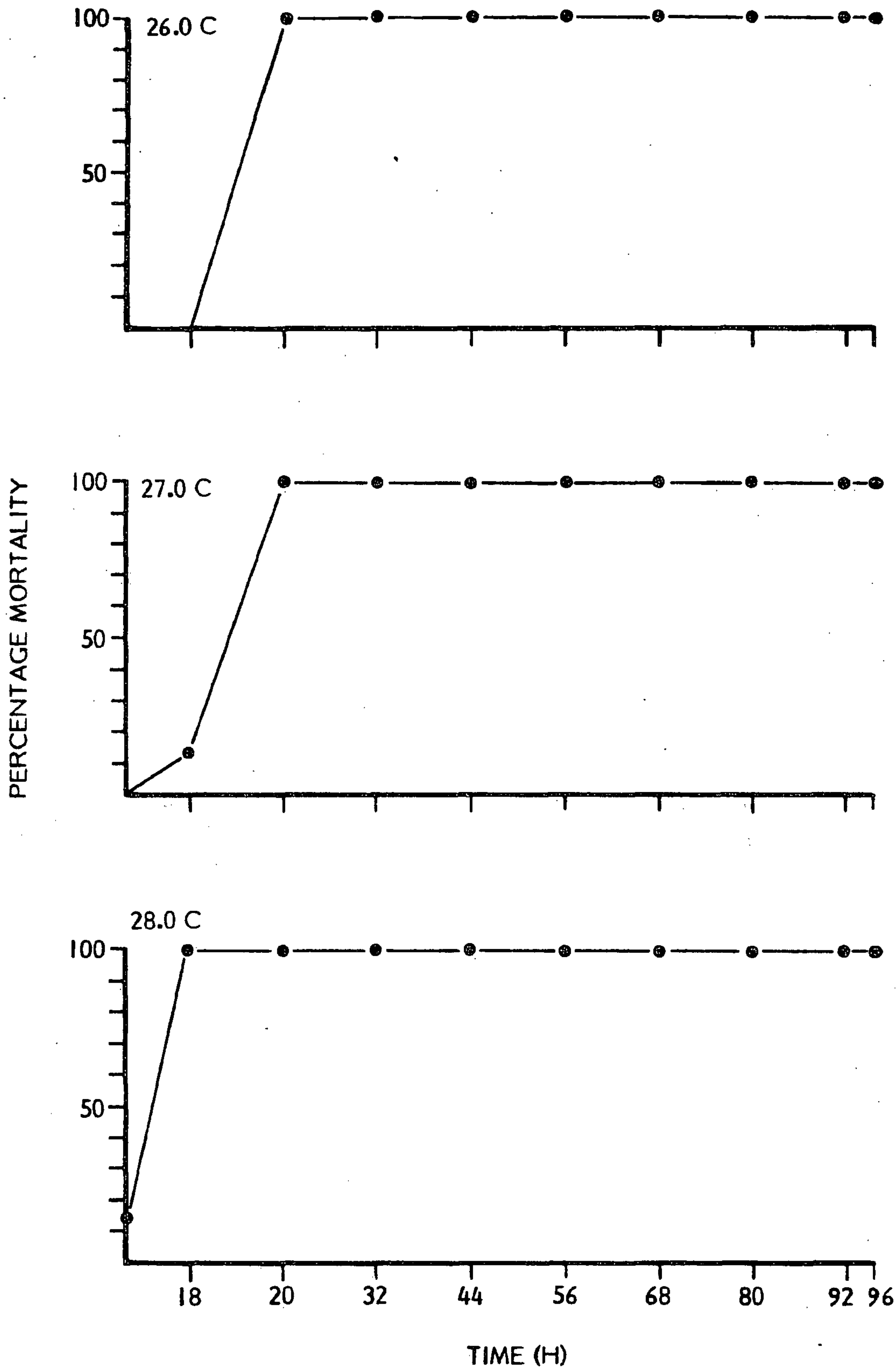

FIGURE 2.10.12-1(cont'd)

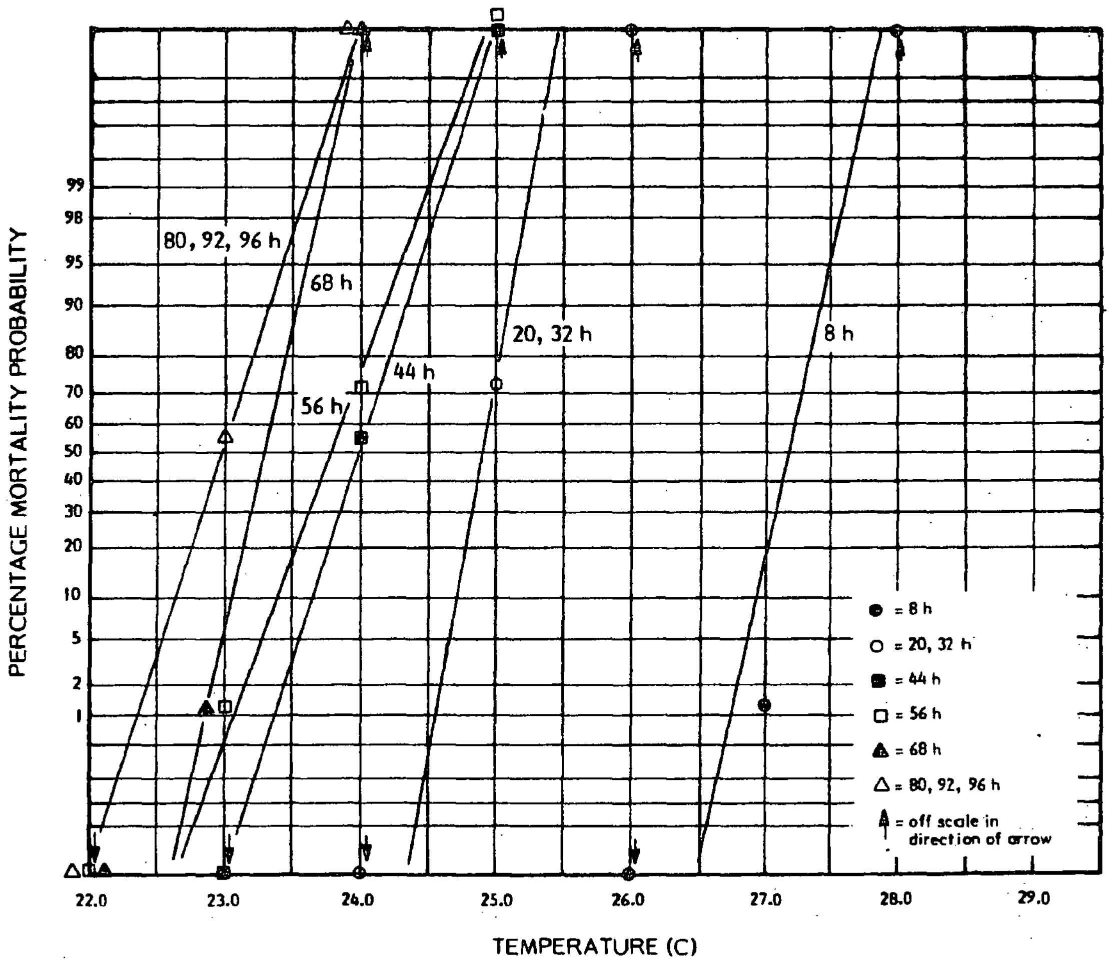

FIGURE 2.10.12-2

PERCENTAGE MORTALITY PROBABILITY VERSUS TEMPERATURE: LEPTASTERIAS HEXACTIS HEAT TOLERANCE

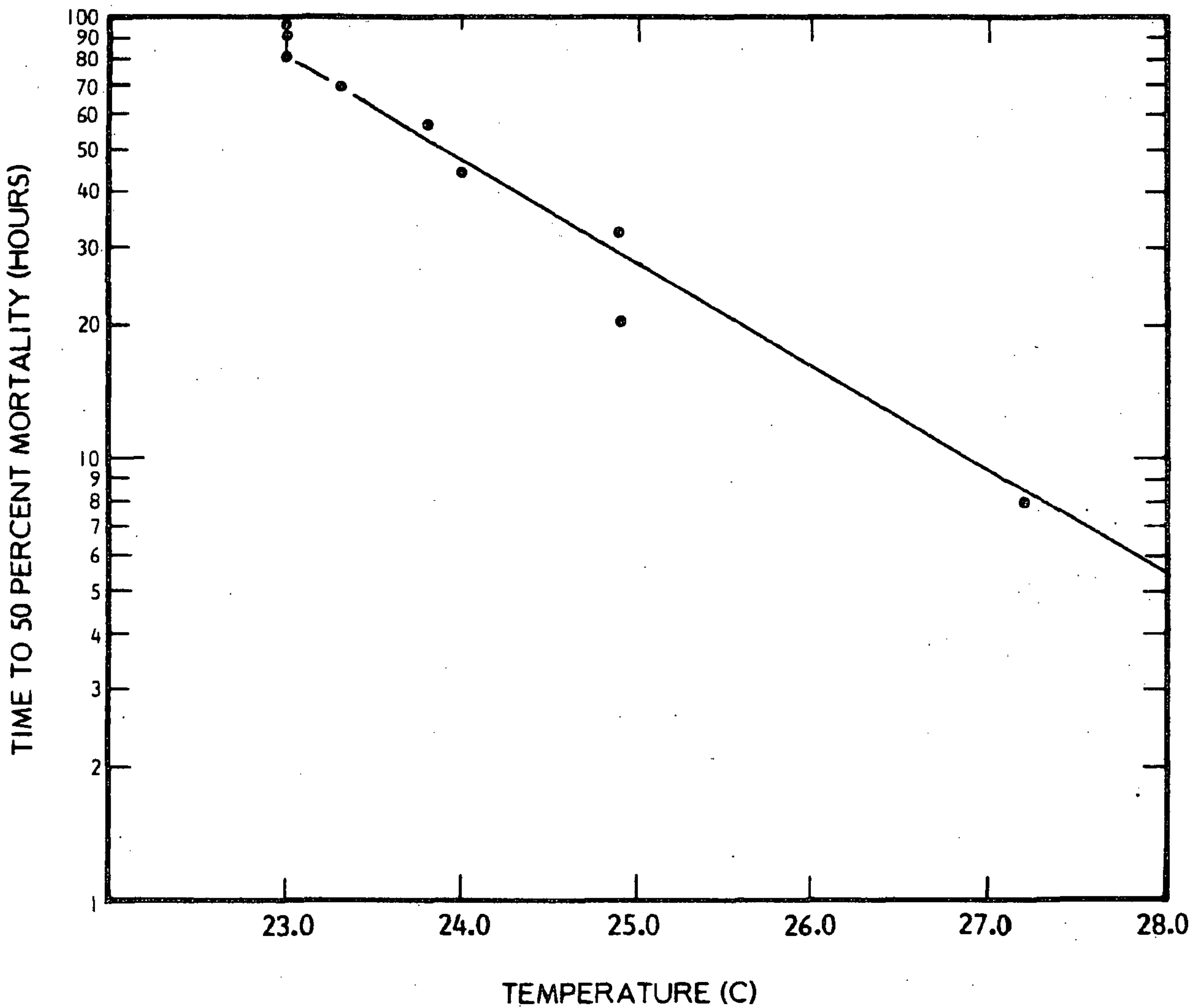

FIGURE 2.10.12-3

TIME TO 50 PERCENT MORTALITY VERSUS TEMPERATURE:
LEPTASTERIAS HEXACTIS HEAT TOLERANCE

2.10.13 OCENEBRA CIRCUMTEXTA 96-HOUR MEDIAN EFFECTIVE TEMPERATURE (96 H ET50)

According to Morris et al. (1980), the circled rock snail, Ocenebra circumtexta, occurs commonly in middle to low intertidal regions in areas of heavy surf. Its geographical distribution extends from Trinidad, Humboldt County, California to Scammon Lagoon in Baja California, Mexico. It is one of numerous murid snails occurring along the central California coast, with shell lengths to about 25 mm.

Although O. circumtexta is not an RIS and has not been recorded in the 316(a) samples as one of the more abundant of the intertidal invertebrates, it is present in the Diablo Cove vicinity and some populations of this species could be exposed to the thermal plume. The species was included in a 96-hour ET50 experiment that was conducted on several RIS because space was available in the tanks and only a minimal increase in effort was necessary to obtain this thermal tolerance information.

2.10.13.1 METHODS

A summary of the methods employed in the heat tolerance experiment on Ocenebra circumtexta was presented in SECTION 2.4.2. A more detailed account of the experimental procedure used in 96-hour ET50 experiments is presented in APPENDIX B (Procedure 319). The protocol for this experiment is included in APPENDIX C (experiment 19c).

The adult specimens of O. circumtexta used in this experiment were collected on 20 June and 12 July 1979 at north "Field's Cove," just north of Diablo Cove. The animals were held in the laboratory at ambient temperatures and were kept continuously submerged throughout their stay in the laboratory. During the holding period, the animals were carefully observed and those which appeared damaged or otherwise unhealthy were excluded from use in the experiment.

The animals were kept in the test and control tanks underneath a slip-fit coupling for 3 in. PVC pipe screened at one end with 1/8 in. mesh Vexar plastic.

These cages were separated from other species run in the same experiment by being placed within a 12 x 18 in. compartment made of 1/8 in. Vexar in a 4 x 8 ft tank. Temperatures were controlled and recorded by an Autodata Nine computer. The temperature data were transcribed from paper tapes onto data sheets and summarized as cumulative means and standard deviations for each observation period.

Observations were made after 6 hours and subsequently every 12 hours for the remainder of the 96-hour test period. Experimental target temperatures were 19, 20.5, 22, 23.5, 25, 26.5 and 28C. An ambient control treatment was also maintained.

The criterion for death was the failure to retract into the shell when gently prodded. Animals that were presumed dead were returned to an ambient temperature tank to assess possible recovery. Mortality data were analyzed with probit analysis in accordance with Standard Methods (1976).

The following species were run simultaneously in this 96-hour test: Acanthina spirata, Cancer antennarius, Collisella digitalis, C. limatula, C. pelta, C. scabra, Hemigrapsus nudus, Pachygrapsus crassipes, Pugettia producta, P. richii, and Strongylocentrotus purpuratus.

2.10.13.2 RESULTS

Cumulative means and standard deviations of temperature data for each observation period are presented in TABLE 2.10.4-1.

Mortality occurred only in the 26.5 and 28.0C tanks. The data on mortality percentage versus time for the two tanks are presented in FIGURE 2.10.13-1. None of the presumed dead animals recovered after being placed in the ambient temperature recovery tank.

Probit regression lines were fitted by eye to the mortality data to determine the temperature which would cause 50 percent mortality at each observation period

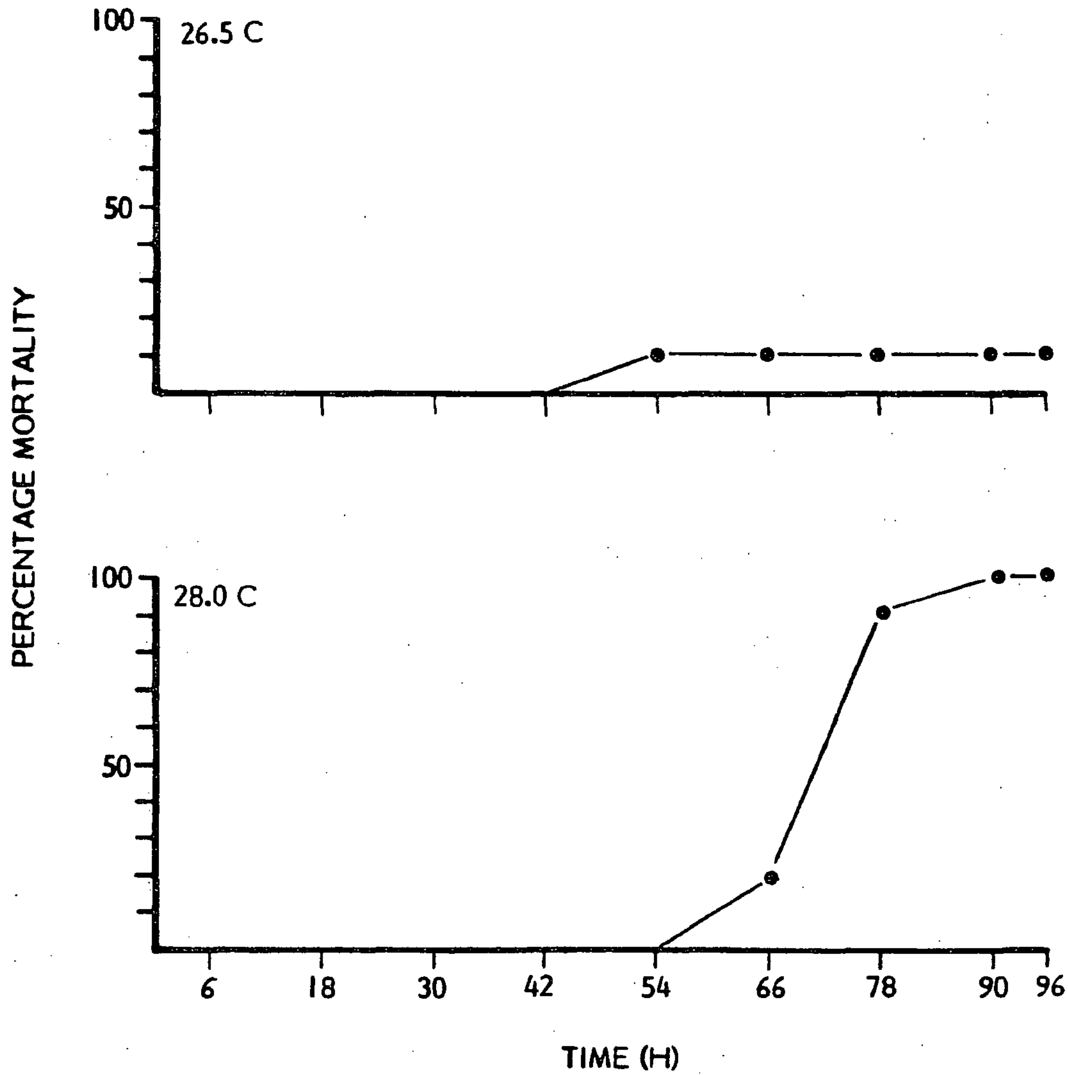

FIGURE 2.10.13-1

CUMULATIVE MORTALITY VERSUS TIME:
OCENEBRA CIRCUMTEXTA HEAT TOLERANCE

(FIGURE 2.10.13-2). The 96-hour ET50 value was determined to be 26.8C. Because of the limited mortality data, no further analysis was attempted.

2.10.13.3 DISCUSSION

Based on the results of this experiment, seawater temperatures in excess of 26.8C (80.2F) for four days would be expected to cause 50 percent mortality in a population of Ocenebra circumtexta. Little or no thermal mortality would be expected for exposures to temperatures at or below 25.0C (77.0F) for the same period of time. Exposure to temperature in excess of 28.0C (82.4F) for 90 hours or more would be expected to cause 100 percent mortality in local populations of O. circumtexta.

However, it should be noted that O. circumtexta occurs in the intertidal region, alternately submerged and exposed to the air, whereas in this experiment thermal exposure was induced by continual submergence. The environmental regime to which O. circumtexta is exposed in nature could have an ameliorative effect on thermal stress responses such as those obtained in this experiment.

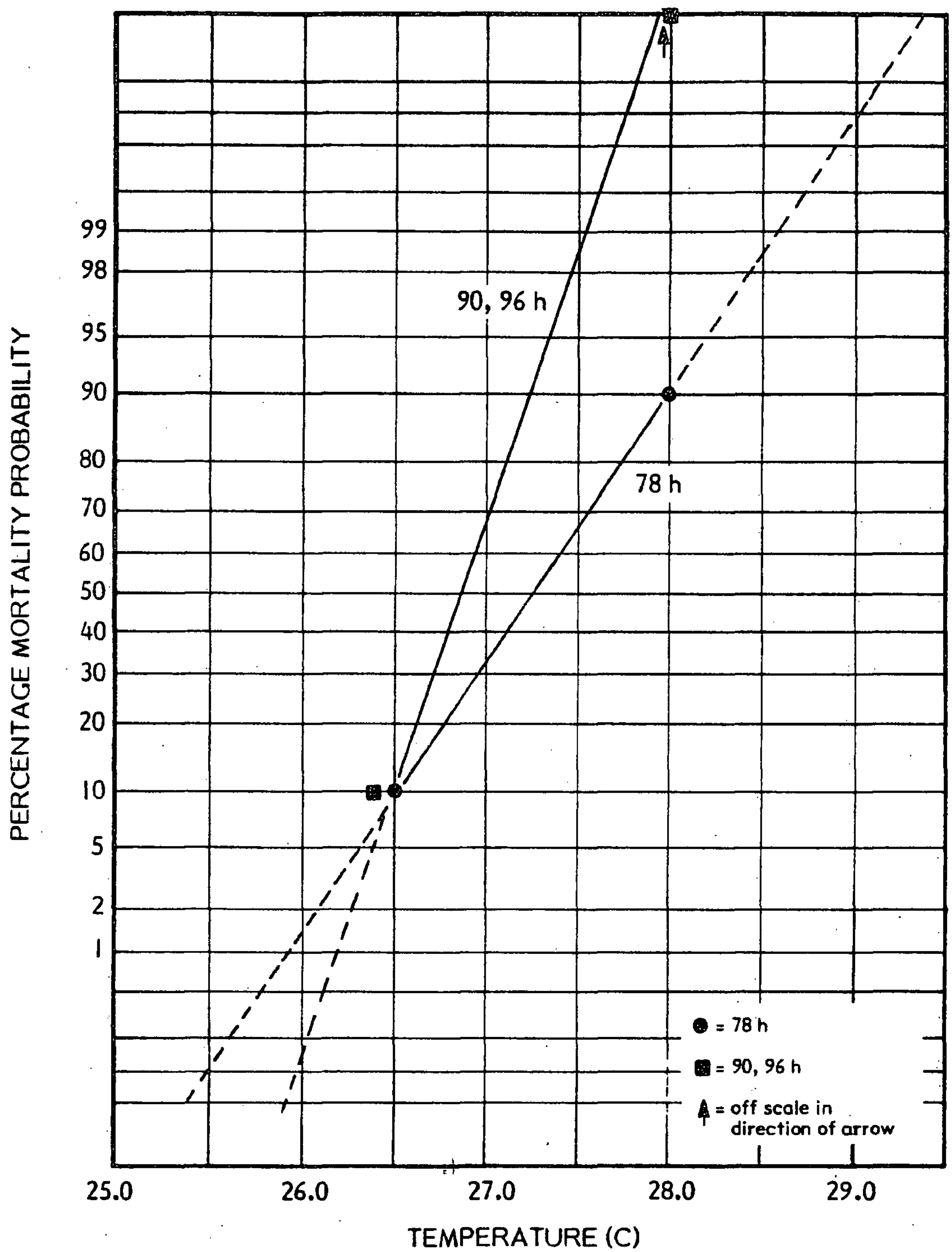

FIGURE 2.10.13-2

PERCENTAGE MORTALITY PROBABILITY VERSUS TEMPERATURE: OCENEBRA CIRCUMTEXTA HEAT TOLERANCE

2.10.14 PACHYGRAPSUS CRASSIPES 96-HOUR MEDIAN EFFECTIVE TEMPERATURE (96 H ET50)

This species, while not an RIS, is a very conspicuous and abundant member of the intertidal region community in the Diablo Canyon study area. The distribution of this shore crab is generally limited to the intertidal region (Ricketts et al. 1968). A review of the literature pertaining to this species indicated no published accounts of its thermal tolerance. The purpose of this experiment was, therefore, to establish the 96-hour ET50 value as an index to the species' ability to tolerate elevated temperatures for prolonged periods.

2.10.14.1 METHODS

A summary of the methods employed in the heat tolerance experiment on this species was presented in SECTION 2.4.2. A more detailed description of the experimental procedure utilized in 96-hour ET50 experiments on invertebrates is presented in APPENDIX B (Procedure 319). The protocol for this experiment is included in APPENDIX C (experiment 19c).

Specimens tested included both juveniles and adults that were collected by hand at low tide. Specimens were acclimated to laboratory conditions at ambient temperature prior to testing. During the experiment crabs were kept completely submerged in test tanks which were subdivided to accommodate several species per test. Temperature was controlled and recorded by an Autodata Nine computer. Temperature data were transcribed and summarized as cumulative means and standard deviations for each observation period.

Observations were made after six hours and subsequently every 12 hours for the 96-hour test period. Experimental target temperatures were 19, 20.5, 22, 23.5, 25, 26.5, and 28 C. An ambient control (mean of 12.5 C) was also run. Test populations consisted of ten animals per treatment.

The criterion for death was the loss of ability to walk when gently prodded. Animals that were presumed dead were returned to an ambient temperature tank to assess possible recovery.

In the first run of the experiment unusually high mortalities occurred in the 26.5 C tank (90 percent after 18 hours). Because these results did not seem comparable with those of other test temperatures, a second run at 26.5 C was initiated. Results of both tests are reported.

2.10.14.2 RESULTS

Cumulative means and standard deviations of temperature data for each observation period are presented in TABLE 2.4-6.

The time course of mortality is presented in FIGURE 2.10.14-1. No mortalities occurred at temperatures below 22 C. At the end of the 96-hour test period, 30 percent of the animals held at 22 and 23.5 C had died, and 40 percent of the test group at 28 C had died. In the first run of the experiment, 90 percent of the test group in the 26.5 C tank died within 18 hours, but upon repeating the experiment at this temperature only 10 percent had died after 96 hours. We are unable to account for the high mortality in the first run, but the results suggest that these were not thermal deaths. None of the animals considered dead in either run recovered after 163 hours at ambient temperature.

Since 50 percent mortality was not attained at any test temperature, further analysis by probit analysis is not warranted. From the results, it appears that the 96-hour ET50 for this species is slightly above 28 C.

2.10.14.3 DISCUSSION

The results of this experiment indicate that populations of the shore crab, <u>Pachygrapsus crassipes</u>, would suffer less than 50 percent mortality upon exposure to seawater temperatures of 28 C (82.4 F) for a period of four days.

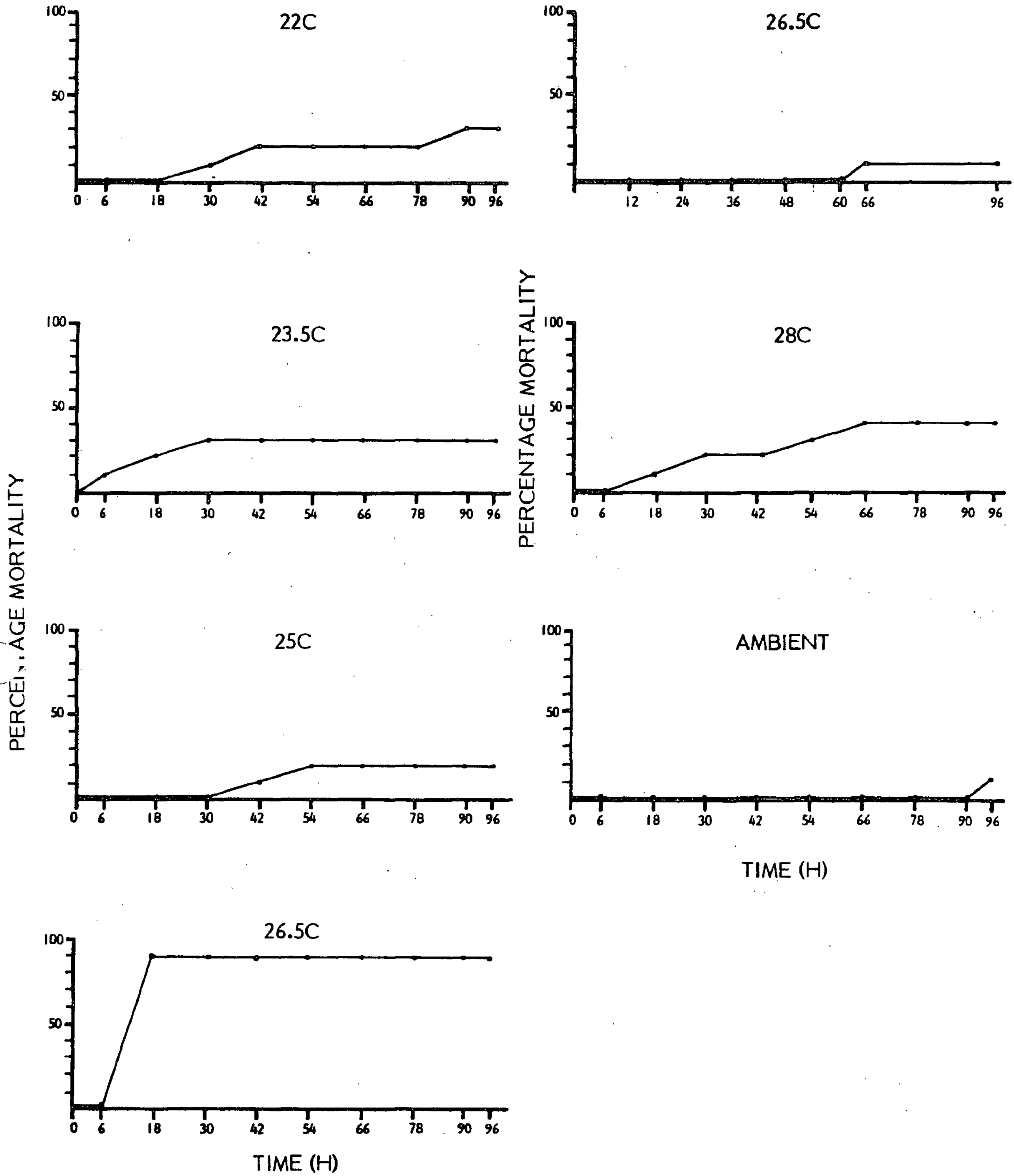

FIGURE 2.10.14-1

CUMULATIVE MORTALITY VERSUS TIME:
PACHYGRAPSUS CRASSIPES HEAT TOLERANCE

The relatively high thermal tolerance of this species is not unexpected considering its distribution in the intertidal region. In this experiment the animals were kept submerged for the duration of the experiment and were thus continually to the test temperature. In their natural habitat shore crabs are frequently subjected to air temperatures in excess of 28 C (82.4 F), but for short durations between tides. While they are probably able to regulate temperature behaviorally, the adaptive significance of a high thermal tolerance to an intertidal invertebrate is apparent.

2.10.15 PAGURUS GRANOSIMANUS 96-HOUR MEDIAN EFFECTIVE TEMPERATURE (96 H ET50)

The hermit crab, Pagurus granosimanus, like its congener, P. samuelis, is an abundant and conspicuous member of the intertidal and shallow subtidal community of the Diablo Canyon study area. This experiment was designed to determine the 96-hour ET50 of this species as an index to its thermal tolerance, since to date no published accounts have been found which deal with this topic. P. granosimanus was tested simultaneously with P. samuelis and the porcelain crab, Petrolisthes cinctipes, in subdivided tanks.

2.10.15.1 METHODS

A summary of the methods employed in the heat tolerance experiment on this species was presented in SECTION 2.4.2. A more detailed description of the experimental procedure utilized in 96-hour ET50 experiments on invertebrates is presented in APPENDIX B (Procedure 319). The protocol for this experiment is included in APPENDIX C (experiment 19b).

Specimens tested included both juveniles and adults that were collected by hand at low tide. Specimens were acclimated to laboratory conditions at ambient temperature prior to testing. During the experiment crabs were kept completely submerged in test tanks which were subdivided to accommodate several species per test. Temperature was controlled and recorded by an Autodata Nine computer. Temperature data were transcribed and summarized as cumulative means and standard deviations for each observation period.

Observations were made after six hours and subsequently every 12 hours for the 96-hour test period. Experimental target temperatures were 22, 23, 24, 25, 26, 27, and 28 C. An ambient control (mean of 14.2 C) was also run. Test populations consisted of ten animals per treatment.

The criterion for death was the loss of ability to walk when gently prodded. Animals that were presumed dead were returned to an ambient temperature tank to assess possible recovery.

2.10.15.2 RESULTS

TABLE 2.10.15-1 presents cumulative means and standard deviations of temperature data for each observation period.

The time course of mortality is presented in FIGURE 2.10.15-1. Deaths occurred only at 27 and 28 C. At 27 C, 20 percent of the test group had expired after 20 hours and 60 percent were dead at the end of 96 hours. In the 28 C tank, 80 percent died after 44 hours and 100 percent were dead after 56 hours. None of the presumed dead animals recovered after being placed in the ambient temperature recovery tank.

Probit regression lines were fitted by eye to mortality data at 44 and 68 hours. These plots are presented in FIGURE 2.10.15-2. The 44-hour ET50 was determined to be 27.5 C, and the 68-hour ET50 was 26 C. The 96-hour value could not be determined graphically, but the data indicate that it is between 26 and 27 C.

2.10.15.3 DISCUSSION

The results of this experiment indicate that seawater temperatures between 26 and 27 C (78.8 and 80.6 F) would cause 50 percent mortality in a population of the hermit crab, *Pagurus granosimanus*, exposed for four days. Temperatures of 26 C (78.8 F) or lower would have essentially no effects on survivorship over a four-day period.

Both species of hermit crabs tested exhibited fairly high tolerance to elevated temperatures, although the tolerance of *P. granosimanus* was slightly less than that of its congener, *P. samuelis*.

TABLE 2.10.15-1

CUMULATIVE TEMPERATURE MEANS AND STANDARD DEVIATIONS (IN PARENTHESES) FOR ALL OBSERVATION PERIODS: PAGURUS GRANOSIMANUS HEAT TOLERANCE EXPERIMENT

These data also apply to 96-hour ET50 tests on Pagurus samuelis and Petrolisthes Cinctipes.

Target Temperature (C)	Observation Period (hours)									
	0	8	20	32	44	56	68	80	92	96
Ambient	13.6 (0.0)	14.4 (0.3)	14.2 (0.2)	14.4 (0.5)	14.4 (0.5)	14.3 (0.5)	14.4 (0.6)	14.5 (0.6)	14.5 (0.6)	14.5 (0.6)
22	22.2 (0.0)	22.2 (0.0)	22.2 (0.0)	22.2 (0.0)	22.2 (0.0)	22.2 (0.0)	22.2 (0.0)	22.2 (0.0)	22.2 (0.0)	22.2 (0.0)
23	23.1 (0.0)	23.1 (0.0)	23.1 (0.0)	23.1 (0.0)	23.1 (0.0)	23.1 (0.0)	23.1 (0.0)	23.1 (0.0)	23.1 (0.0)	23.1 (0.0)
24	23.9 (0.0)	24.0 (0.0)	24.0 (0.0)	23.9 (0.0)	24.0 (0.0)	23.9 (0.0)	23.9 (0.0)	24.0 (0.0)	24.0 (0.0)	24.0 (0.0)
25	25.0 (0.0)	25.0 (0.0)	25.0 (0.0)	25.0 (0.0)	25.0 (0.0)	25.0 (0.0)	25.0 (0.0)	25.0 (0.0)	25.0 (0.0)	25.0 (0.0)
26	25.4 (0.0)	25.7 (0.0)	25.7 (0.0)	25.7 (0.0)	25.7 (0.1)	25.7 (0.1)	25.7 (0.1)	25.7 (0.1)	25.7 (0.1)	25.7 (0.1)
27	27.0 (0.0)	27.0 (0.0)	27.1 (0.0)	27.0 (0.0)	27.0 (0.0)	27.0 (0.0)	27.0 (0.0)	27.0 (0.0)	27.0 (0.0)	27.0 (0.0)
28	28.0 (0.0)	27.9 (0.0)	28.0 (0.1)	28.0 (0.1)	28.0 (0.0)	28.0 (0.0)	28.0 (0.0)	28.0 (0.1)	27.9 (0.2)	27.9 (0.2)

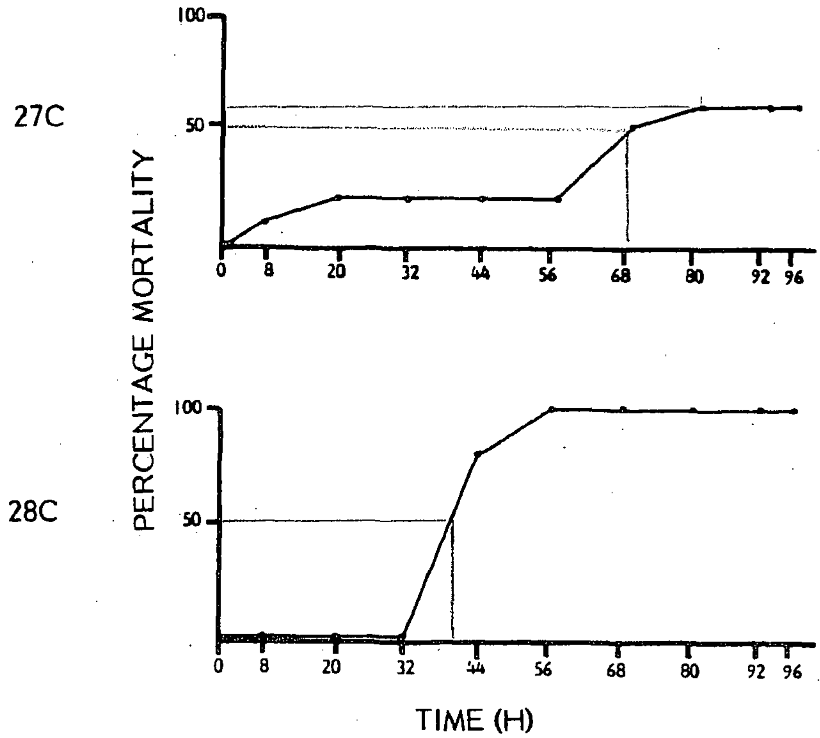

FIGURE 2.10.15-1

CUMULATIVE MORTALITY VERSUS TIME: PAGURUS GRANOSIMANUS HEAT TOLERANCE

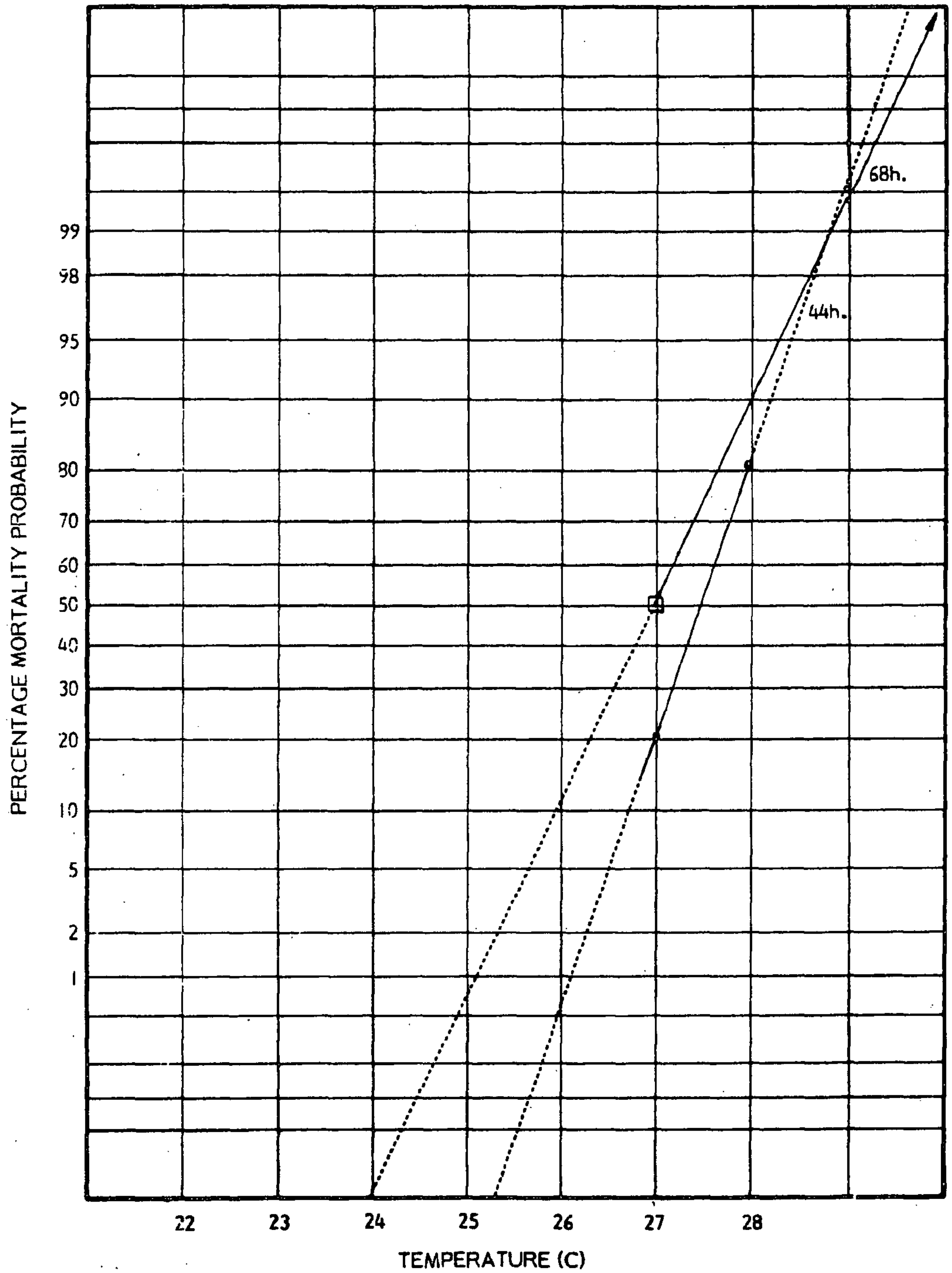

FIGURE 2.10.15-2

PERCENTAGE PROBABILITY MORTALITY VERSUS TEMPERATURE:
PAGURUS GRANOSIMANUS HEAT TOLERANCE

2.10.16. PAGURUS SAMUELIS 96-HOUR MEDIAN EFFECTIVE TEMPERATURE (96 H ET50)

The hermit crab, Pagurus samuelis is very abundant in the intertidal and subtidal regions of the Diablo Canyon study area. This experiment was designed to determine its 96-hour ET50, since no published accounts have been found to date which deal with its thermal tolerance. This species was tested in subdivided tanks concurrently with the congener, P. granosimanus, and the porcelain crab, Petrolisthes cinctipes.

2.10.16.1 METHODS

A summary of the methods employed in the heat tolerance experiment on this species was presented in SECTION 2.4.2. A more detailed description of the experimental procedure utilized in 96-hour ET50 experiments on invertebrates is presented in APPENDIX B (Procedure 319). The protocol for this experiment is included in APPENDIX C (experiment 19c).

Specimens tested included both juveniles and adults that were collected by hand at low tide. Specimens were acclimated to laboratory conditions at ambient temperature prior to testing. During the experiment crabs were kept completely submerged in test tanks which were subdivided to accommodate several species per test. Temperature was controlled and recorded by an Autodata Nine computer. Temperature data were transcribed and summarized as cumulative means and standard deviations for each observation period.

Observations were made after 6 hours and subsequently every 12 hours for the 96-hour test period. Experimental temperatures were 22, 23, 24, 25, 26, 27 and 28 C. An ambient control (mean of 14.2 C) was also run. Test populations consisted of ten animals per treatment.

The criterion for death was the loss of ability to walk when gently prodded. Animals that were presumed dead were returned to an ambient temperature tank to assess possible recovery.

2.10.16.2 RESULTS

Cumulative means and standard deviations of temperature data for each observation period are presented in TABLE 2.10.15-1.

FIGURE 2.10.16-1 presents the time course of mortality. After 96 hours, 20 percent mortality occurred at 26 and 27 C, and 40 percent mortality was observed at 28 C. None of the animals that were presumed dead recovered after replacement in the ambient temperature recovery tank. Since 50 percent mortality did not occur at any temperature, the 96-hour ET50 could not be determined. Based on the results, however, the value is probably slightly above 28 C.

2.10.16.3 DISCUSSION

Based on the results of this experiment, seawater temperatures slightly in excess of 28 C (82.4 F) would probably result in 50 percent mortality of populations of the hermit crab Pagurus samuelis exposed for four day periods. Exposures to temperatures below 26 C (78.8 F) for the same period would have little or no effect on survivorship.

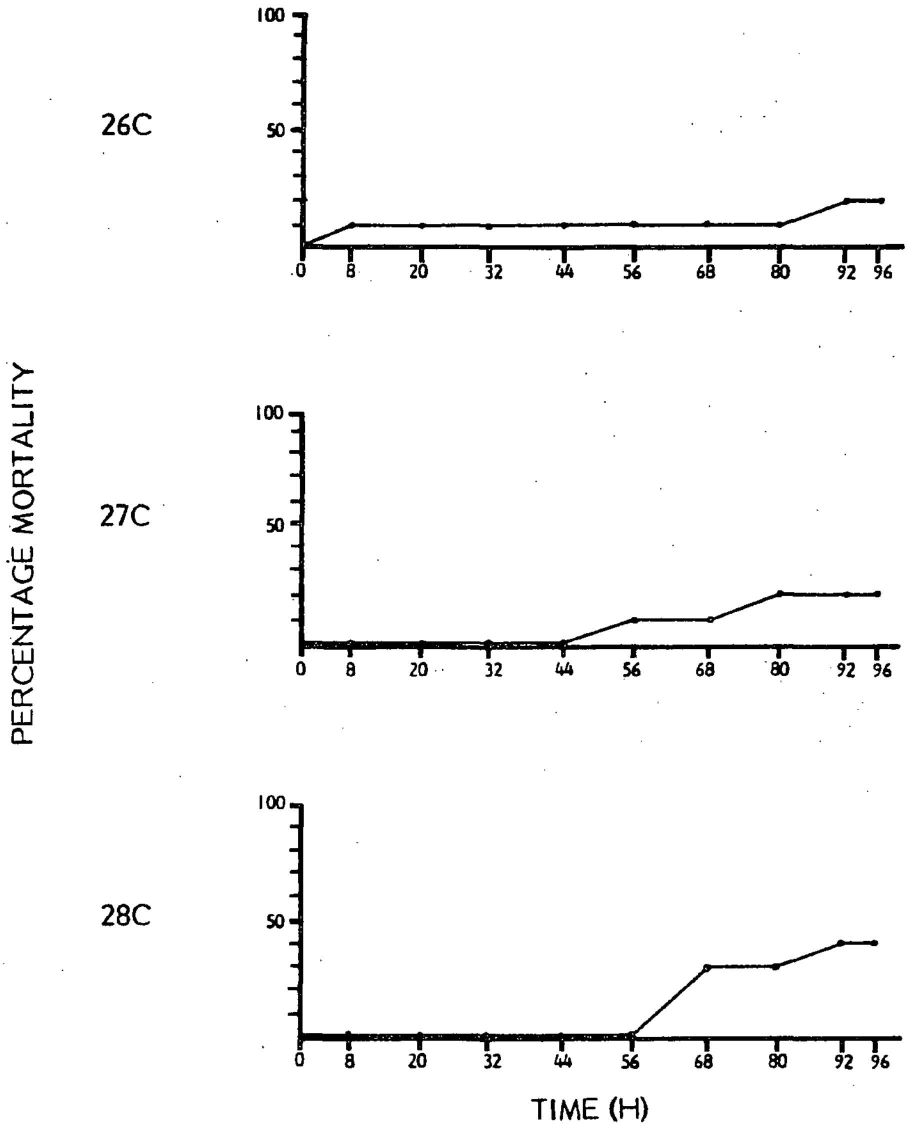

FIGURE 2.10.16-1

CUMULATIVE MORTALITY VERSUS TIME:
PAGURUS SAMUELIS HEAT TOLERANCE

2.10.17 PATIRIA MINIATA 96-HOUR MEDIAN EFFECTIVE TEMPERATURE (96H ET50)

According to Morris et al. (1980), the bat star or sea bat, Patiria miniata, occurs commonly in central California among rocks overgrown with surfgrass, larger algae, sponges and bryozoans in the lower intertidal zone. It extends into the subtidal to 290 m (950 ft) depth on various substrata. Geographically, the species extends from Sitka, Alaska to Baja California, Mexico. P. miniata is typically an omnivore and scavenger that feeds on a great variety of plants and animals (Morris et al., 1980).

Although P. miniata is not an RIS, it was found to be a "ubiquitous taxon" on the ¼ x ¼ m fixed subtidal quadrats and on the 30-m^2 arc quadrats. It was present at all 15-ft stations and at four of five 10-ft stations (PGandE 1979a). It is thus likely that some populations of this species could be exposed to the thermal plume. The species was included in a 96-hour ET50 experiment that was conducted on several RIS because space was available in the tanks and only a minimal increase in effort was necessary to obtain this thermal tolerance information.

2.10.17.1 METHODS

A summary of the methods employed in the heat tolerance experiment on Patiria miniata was presented in SECTION 2.4.2. A more detailed account of the experimental procedure used in 96-hour ET50 experiments is presented in APPENDIX B (Procedure 319). The protocol for this experiment is included in APPENDIX C (experiment 19b).

The adult specimens of P. miniata used in this experiment were collected on 18 April 1979 just south of Diablo Cove. The animals were held in the laboratory at ambient temperatures and were kept continuously submerged throughout their stay in the laboratory. During the holding period, the animals were carefully observed and those which appeared damaged or otherwise unhealthy were excluded from use in the experiment.

The animals were kept in the test and control tanks in 2 ft x 18 in. compartments made of 1/8 in. mesh Vexar screen attached to 1 in. PVC pipe frame with a fiberglass sheet bottom. The animals were continuously submerged during the course of the experiment. Temperatures were controlled and recorded by an Autodata Nine computer. The temperature data were transcribed from paper tapes onto data sheets and summarized as cumulative means and standard deviations for each observation period.

Observations were made after 8 hours and subsequently every 12 hour for the remainder of the 96-hour test period. Experimental target temperatures were 22, 23, 24, 25, 26, 27 and 28C. An ambient control treatment was also maintained. The number of animals tested was 10 per treatment and control.

The criteria for death were the lack of ability to hold onto a surface and/or the absence of tube feet movement. Animals that were presumed dead were returned to an ambient temperature tank to assess possible recovery. Animals remaining alive at the end of the 96-hour test period were returned to ambient temperature. After about 72 hour (167 hours after the start of the 96-hour test) the animals were observed to determine if any "latent" mortality occurred. Mortality data were analyzed with probit analysis in accordance with Standard Methods (1976).

The following species were run simultaneously in this 96-hour test: Leptasterias hexactis, Pagurus granosimanus, P. samuelis, Petrolisthes cinctipes, Pisaster brevispinus, P. giganteus and P. ochraceus.

2.10.17.2 RESULTS

Cumulative means and standard deviations of temperature data for each observation period are presented in TABLE 2.10.12-1.

Mortality occurred in the 27 and 28C tanks. Plots of the mortality percentage versus time for each of these treatments are presented in FIGURE 2.10.17-1. None of the presumed dead animals recovered after being placed in the ambient

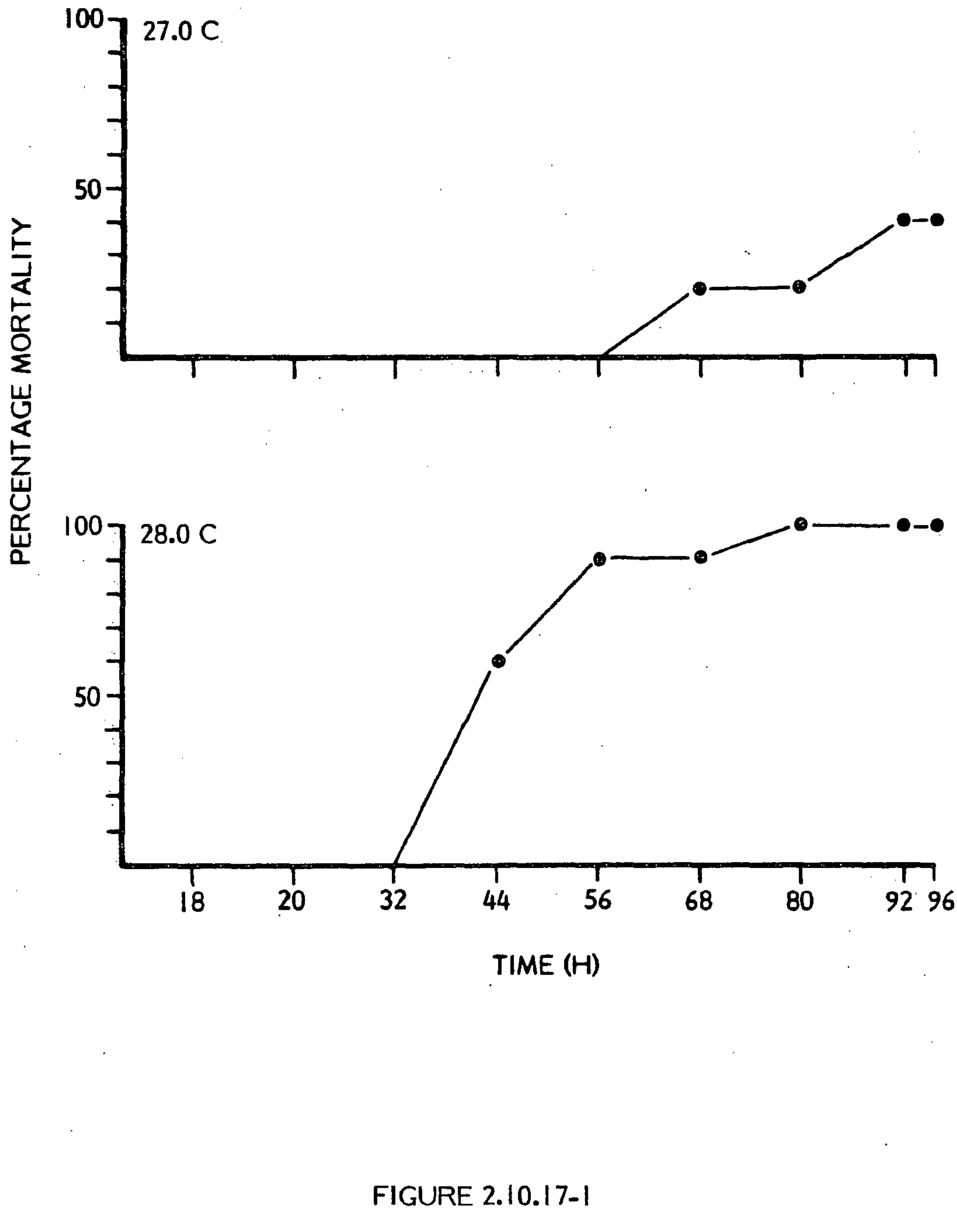

FIGURE 2.10.17-1

CUMULATIVE MORTALITY VERSUS TIME:
PATIRIA MINIATA HEAT TOLERANCE

temperature recovery tank. None of the animals that were alive at the end of the 96-hour period showed "latent" mortality. The 96-hour ET50 value was not precisely determined but lies between 27 and 28C. Because of the limited mortality data, no further analyses were conducted.

2.10.17.3 DISCUSSION

Based on the results of this experiment, seawater temperatures in excess of 27-28C (81-82F) for four days would be expected to cause at least 50 percent mortality in a population of Patiria miniata. Little or no thermal mortality would be expected for exposures to temperatures at or below 26C (79F) for the same period of time. Exposure to 28C (82F) for 80 hour would be expected to cause 100 percent mortality in a population of P. miniata. However, it should be noted that the intertidal populations of P. miniata are alternately submerged and exposed to the air, whereas in this experiment thermal exposure occurred during continual submergence. The environmental regime to which P. miniata is exposed in nature could have an ameliorative effect on the thermal stress responses obtained in this experiment.

2.10.18 PETROLISTHES CINCTIPES 96-HOUR MEDIAN EFFECTIVE TEMPERATURE (96 H ET50)

The porcelain crab, Petrolisthes cinctipes, is very abundant in the Diablo Canyon study area. It is not as conspicuous as many of the other intertidal crab species because of its cryptic nature. It is commonly found beneath rocks and boulders in the middle intertidal zone. There are no published accounts of the thermal tolerance of this species. The purpose of this experiment was, therefore, to determine the 96-hour ET50 of this species as an index to its thermal tolerance. This species was tested simultaneously with the hermit crabs Pagurus samuelis and P. granosimanus in subdivided tanks.

2.10.18.1 METHODS

A summary of the methods employed in the heat tolerance experiment on this species was presented in SECTION 2.4.2. A more detailed description of the experimental procedure utilized in 96-hour ET50 experiments on invertebrates is presented in APPENDIX B (Procedure 319). The protocol for this experiment is included in APPENDIX C (experiment 19b).

Specimens tested included both juveniles and adults that were collected by hand at low tide. Specimens were acclimated to laboratory conditions at ambient temperature prior to testing. During the experiment crabs were kept completely submerged in test tanks which were subdivided to accommodate several species per test. Temperature was controlled and recorded by an Autodata Nine computer. Temperature data were transcribed and summarized as cumulative means and standard deviations for each observation period.

Observations were made after six hours and subsequently every 12 hours for the 96-hour test period. Experimental target temperatures were 22, 23, 24, 25, 26, 27, and 28 C. An ambient control (mean of 14.2 C) was also run. Test populations consisted of ten animals per treatment.

The criterion for death was the loss of ability to walk and/or lack of antennule movement when gently prodded. Animals that were presumed dead were returned to an ambient temperature tank to assess possible recovery. Mortality data were analyzed with probit analysis in accordance with Standard Methods (1976).

2.10.18.2 RESULTS

TABLE 2.10.15-1 presents cumulative means and standard deviations of temperature data for each observation period.

FIGURE 2.10.18-1 presents the time course of mortality. Deaths occurred only at 27 and 28 C. At 27 C 18 percent of the test population had died at the end of 96 hours while at 28 C 50 percent had died at 92 hours and 60 percent had died after 96 hours. None of the presumed dead animals recovered after being placed in the ambient temperature recovery tank.

Probit regression lines were fitted by eye to the mortality data (FIGURE 2.10.18-2). The 96-hour ET50 value determined from this procedure was 27.7 C. Since an ET50 value could only be determined for the 96-hour observation period, the "incipient lethal" temperature could not be determined.

2.10.18.3 DISCUSSION

Using the criterion of inability to walk when prodded and/or lack of antennule movement to determine death, seawater temperatures of 27.7 C (81.9 F) would be expected to cause 50 percent mortality of a population of the porcelain crab, Petrolisthes cinctipes, exposed for four days. Exposure for the same period to temperatures of 26 C (78.8 F) or less would be expected to cause little or no mortality.

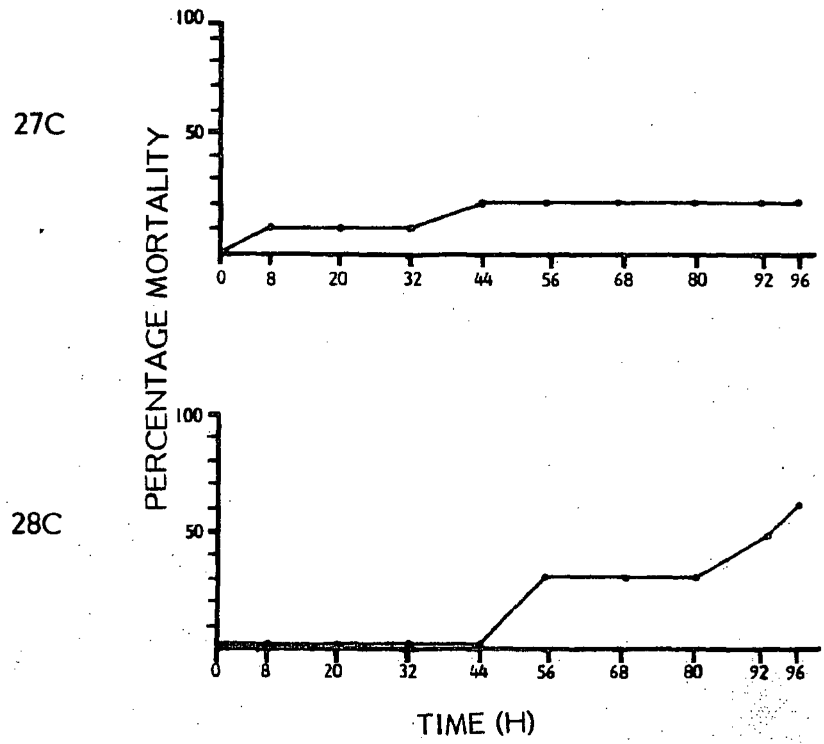

FIGURE 2.10.18-1

CUMULATIVE MORTALITY VERSUS TIME:
PETROLISTHES CINCTIPES HEAT TOLERANCE

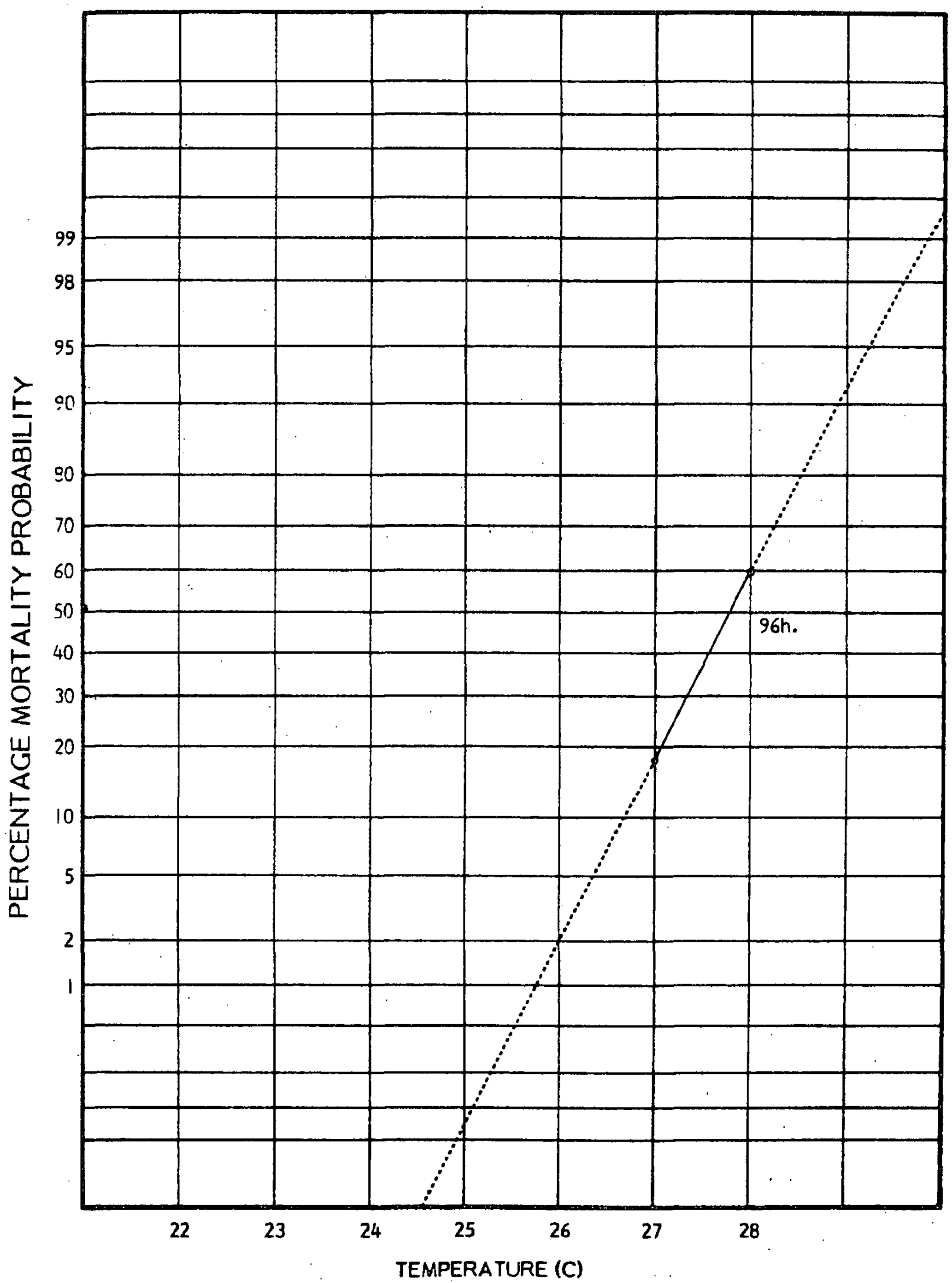

FIGURE 2.10.18-2

PERCENTAGE PROBABILITY MORTALITY VERSUS TEMPERATURE: PETROLISTHES CINCTIPES HEAT TOLERANCE

2.10.19 PISASTER BREVISPINUS 96 HOUR MEDIAN EFFECTIVE TEMPERATURE (96 H ET50)

According to Morris et al. (1980), Pisaster brevispinus, one of the largest of all sea stars, occurs only occasionally in the lower intertidal zone. It is adapted to the subtidal region, being highly sensitive to dessication, and ranges to depths of 100 m (325 ft), primarily on sandy and muddy bottom. Geographically, P. brevispinus ranges from Sitka, Alaska to Mission Bay, San Diego County, California. P. brevispinus is primarily a carnivore. It is congeneric with the RIS P. ochraceus.

Although P. brevispinus is not an RIS and has not been recorded in the 316(s) samples as one of the more abundant of the intertidal invertebrates, it is present in the Diablo Cove vicinity and some populations of this species could be exposed to the thermal plume. The species was included in a 96-hour ET50 experiment that was conducted on several RIS because space was available in the tanks and only a minimal increase in effort was necessary to obtain this thermal tolerance information.

2.10.19.1 METHODS

A summary of the methods employed in the heat tolerance experiment on Pisaster brevispinus was presented in SECTION 2.4.2. A more detailed account of the experimental procedure used in 96-hour ET50 experiments is presented in APPENDIX B (Procedure 319). The protocol for this experiment is included in APPENDIX C (experiment 19b).

The adult specimens of P. brevispinus used in this experiment were collected from June to July 1979 in the vicinity of Diablo Cove. The animals were held in the laboratory at ambient temperatures and were kept continuously submerged throughout their stay in the laboratory. During the holding period, the animals were carefully observed and those which appeared damaged or otherwise unhealthy were excluded from use in the experiment.

The experiment was carried out in 4 x 8 x 2 ft tanks, each of which (except the ambient tank) was programmed to a test temperature. Large specimens of P. brevispinus were held in the tanks inside 2 x 3 ft x 18 in. cages fabricated of 1/8 in. mesh Vexar screen attached to ½ in. PVC pipe frame with a fiberglass sheet bottom. Small specimens were held with Leptasterias hexactis in 5-gal white buckets on concrete blocks. Each bucket was supplied with a standpipe to maintain the water level just below the rim and with water and air supply hoses. The air line was connected to an airstone. The animals were continuously submerged during the course of the experiment. Temperatures were controlled and recorded by an Autodata Nine computer. The temperature data were transcribed from paper tapes onto data sheets and summarized as cumulative means and standard deviations for each observation period.

Observations were made after 8 hours and subsequently every 12 hours for the remainder of the 96-hour test period. Experimental target temperatures were 22, 23, 24, 25, 26, 27 and 28C. An ambient control treatment was also maintained. The number of animals tested was seven for the ambient and eight each for the test temperature treatments.

The criteria for death were the lack of ability to hold onto a surface and/or the absence of tube feet movement. Animals that were presumed dead were returned to an ambient temperature tank to assess possible recovery. Animals remaining alive at the end of the 96-hour test period were returned to ambient temperature. After about 72 hours (167 hours after the start of the 96-hour test) the animals were observed to determine if any "latent" mortality occurred. Mortality data were analyzed with probit analysis in accordance with Standard Methods (1976).

The following species were run simultaneously in this 96-hour test: Leptasterias hexactis, Pagurus granosimanus, P. samuelis, Patiria miniata, Petrolisthes cinctipes, Pisaster giganteus and P. ochraceus.

2.10.19.2 RESULTS

Cumulative means and standard deviations of temperature data for each observation period are presented in TABLE 2.10.12-1.

Mortality occurred in the 25, 26, 27 and 28C tanks. Plots of the mortality percentage versus time for each of these treatments are presented in FIGURE 2.10.19-1. Of the presumed dead animals, one, from the 27C tank, recovered after being placed in the ambient temperature recovery tank. Because it is not known which of the animals recorded as presumed dead in the test tank recovered, the mortality data were not altered to reflect this fact. All other presumed dead animals did not recover. Following the 96-hour test period, three "latent" mortalities occurred among the animals from the 26C tank for a total of 100 percent mortality.

Probit regression lines were fitted by eye to the mortality data to determine the temperature which would cause 50 percent mortality at each observation period (FIGURE 2.10.19-2). As shown in FIGURE 2.10.19-1 only one animal died in the 25C tank at 20 hour. Because no further mortality occurred at this temperature for the remainder of the 96-hour test period, it appears likely that this death is not due solely to thermal stress. This is supported by the relative position of this data point on the graph of FIGURE 2.10.19-2. For these reasons, this data point was ignored in fitting the lines of FIGURE 2.10.19-2 by hand. The 96-hour ET50 value was determined to be approximately 25.8C. The estimators of the ET50 value thus calculated for each observation period were used to generate a curve of time to 50 percent mortality versus temperature, which is presented in FIGURE 2.10.19-3. The "incipient lethal" temperature, indicated by the vertical alignment of points to the left of the curve, was not determined but appears to be somewhat below 26C.

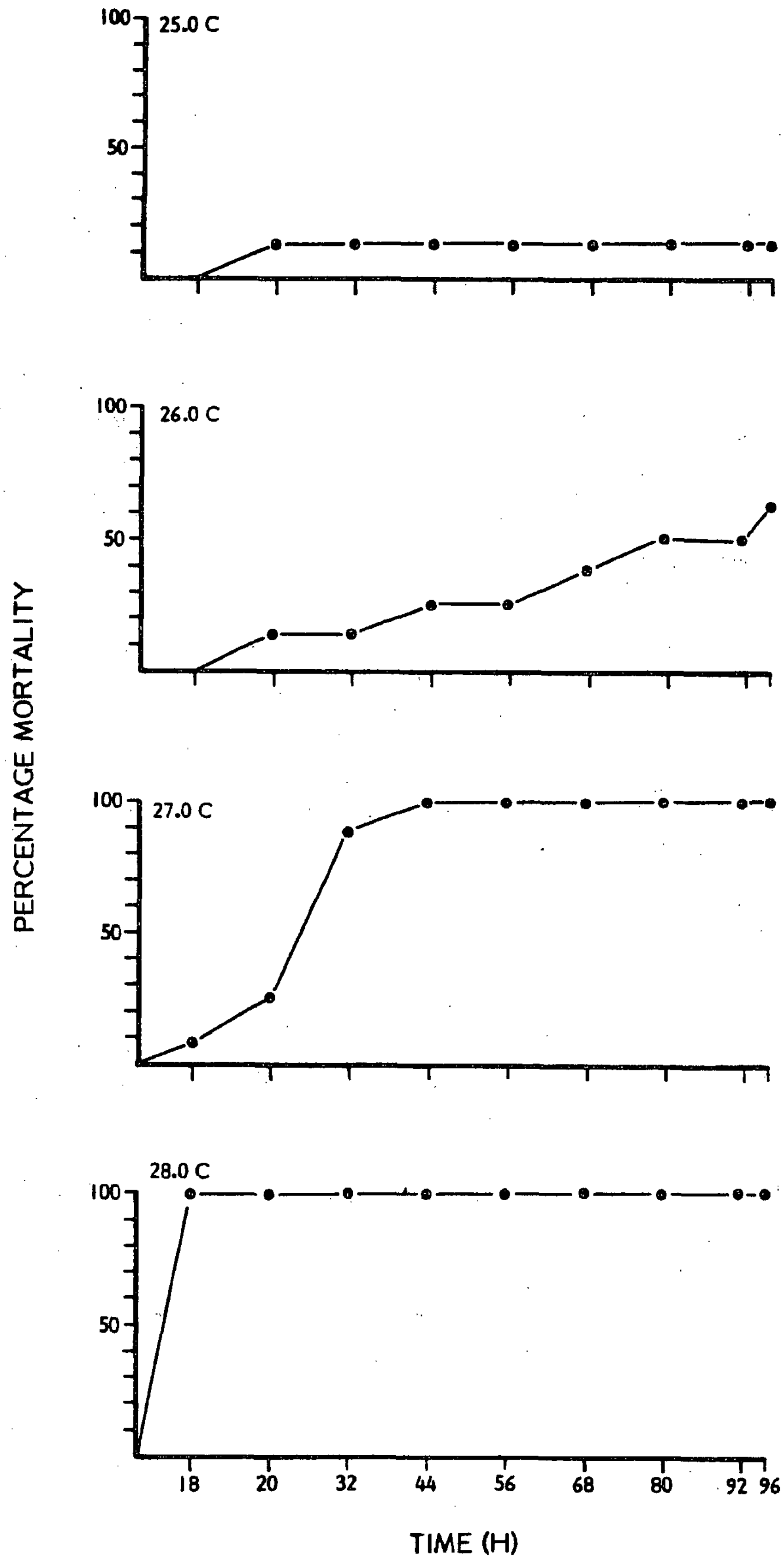

FIGURE 2.10.19-1

CUMULATIVE MORTALITY VERSUS TIME:
PISASTER BREVISPINUS HEAT TOLERANCE

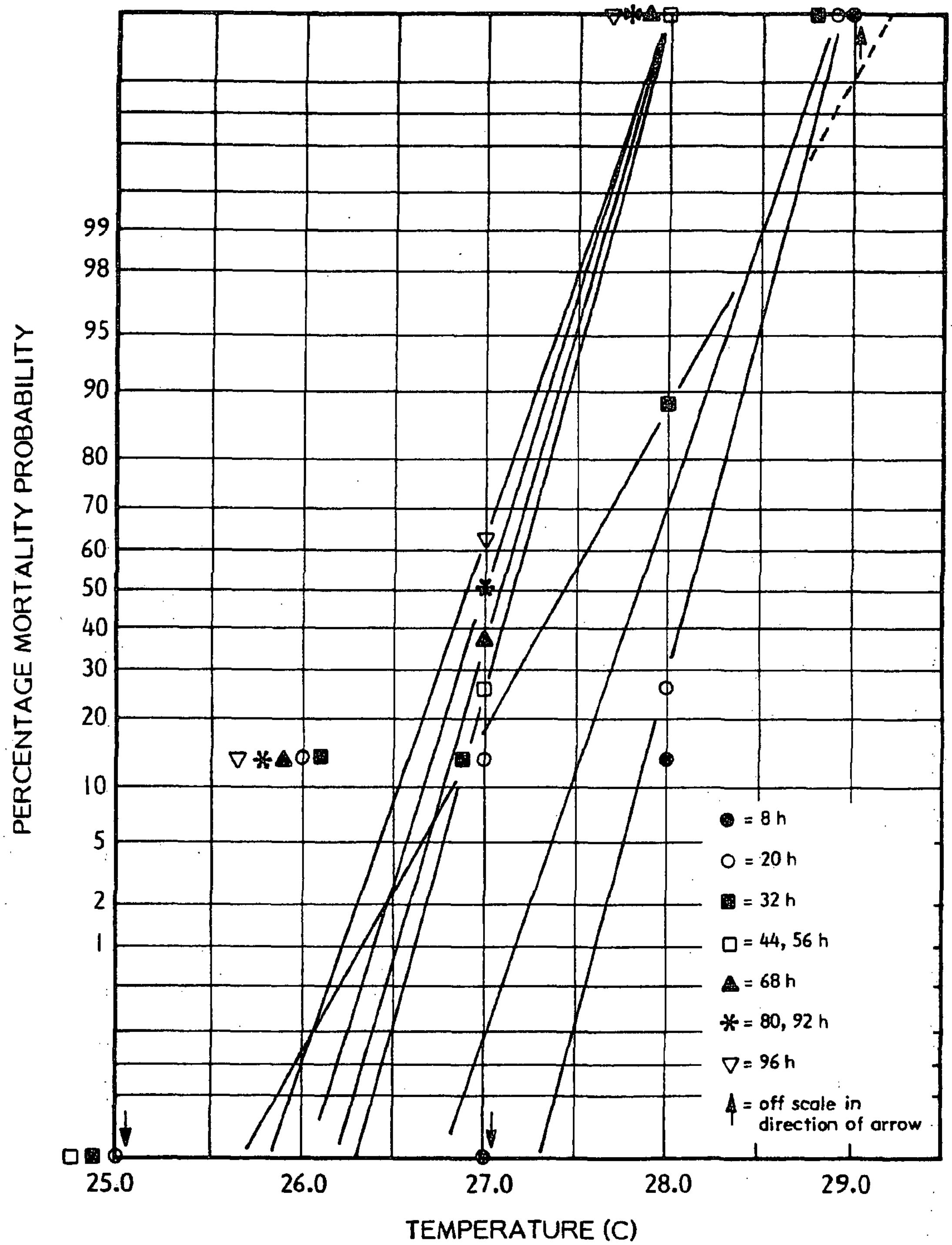

FIGURE 2.10.19-2

PERCENTAGE MORTALITY PROBABILITY VERSUS TEMPERATURE: PISASTER BREVISPINUS HEAT TOLERANCE

The lines were fitted by eye, ignoring the single mortality at 25 C.

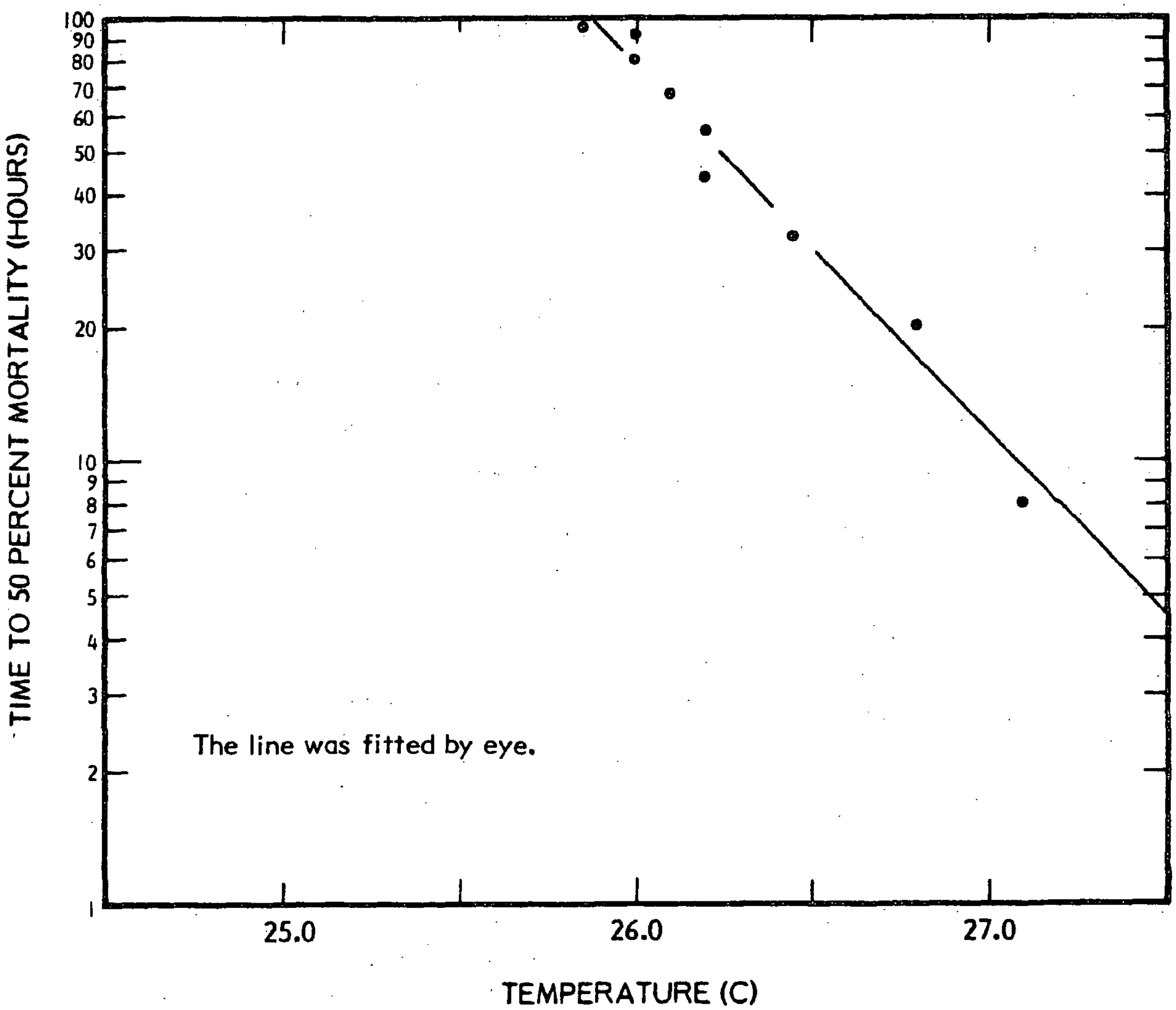

FIGURE 2.10.19-3

TIME TO 50 PERCENT MORTALITY VERSUS TEMPERATURE: PISASTER BREVISPINUS HEAT TOLERANCE

2.10.19.3 DISCUSSION

Based on the results of this experiment, seawater temperatures in excess of 25.8C (78.4F) for four days would be expected to cause 50 percent mortality in a population of Pisaster brevispinus. Little or no thermal mortality would be expected for exposures to temperatures at or below 25.0C (71.6F) for the same period of time. Exposure to 27C (81F) for 44 hours and to 28C (82F) for 8 hours would be expected to cause 100 percent mortality in a population of P. brevispinus.

It should be noted that P. brevispinus occurs almost entirely in the subtidal region, and the major portion of its population would occur below the thermal plume.

2.10.20 PISASTER GIGANTEUS 96-HOUR MEDIAN EFFECTIVE TEMPERATURE (96 H ET50)

According to Morris et al. (1980), Pisaster giganteus occurs commonly on rocky shores in the very low intertidal zone to 88 m (288 ft) depth in protected coastal areas. Geographically, the species extends from Vancouver Island, British Columbia, Canada to Baja California, Mexico. It is one of the largest Pacific sea stars, with an arm radius up to 30 cm. P. giganteus is a carnivore that feeds on prey such as bivalves, snails, chitons and barnacles.

Although P. giganteus is not an RIS, it was a "ubiquitous taxon" on the subtidal 30-m^2 arc quadrats (PGandE 1979a). Thus, some populations of this species could be exposed to the thermal plume. The species was included in a 96-hour ET50 experiment that was conducted on several RIS because space was available in the tanks and only a minimal increase in effort was necessary to obtain this thermal tolerance information.

2.10.20.1 METHODS

A summary of the methods employed in the heat tolerance experiment on Pisaster giganteus was presented in SECTION 2.4.2. A more detailed account of the experimental procedure used in 96-hour ET50 experiments is presented in APPENDIX B (Procedure 319). The protocol for this experiment is included in APPENDIX C (experiment 19b).

The adult specimens of P. giganteus used in this experiment were collected from April to July 1979 just south of Diablo Cove. The animals were held in the laboratory at ambient temperatures and were kept continuously submerged throughout their stay in the laboratory. During the holding period, the animals were carefully observed and those which appeared damaged or otherwise unhealthy were excluded from use in the experiment.

The experiment was carried out in 4 x 8 x 2 ft tanks, each of which (except the ambient tank) was programmed to a test temperature. Large specimens of P.

giganteus were held in the tanks inside 2 x 3 ft x 18 in. cages fabricated of 1/8 in. mesh Vexar screen attached to ½ in. PVC pipe frame with a fiberglass sheet bottom. The animals were continuously submerged during the course of the experiment. Temperatures were controlled and recorded by an Autodata Nine computer. The temperature data were transcribed from paper tapes onto data sheets and summarized as cumulative means and standard deviations for each observation period.

Observations were made after 8 hours and subsequently every 12 hours for the remainder of the 96-hour test period. Experimental target temperatures were 22, 23, 24, 25, 26, 27 and 28C. An ambient control treatment was also maintained. The number of animals tested was 10 each for the ambient and test tanks.

The criteria for death were the lack of ability to hold onto a surface and/or the absence of tube feet movement. Animals that were presumed dead were returned to an ambient temperature tank to assess possible recovery. Animals remaining alive at the end of the 96-hour test period were returned to ambient temperature. After about 72 hours (167 hours after the start of the 96-hour test) the animals were observed to determine if any "latent" mortality occurred. Mortality data were analyzed with probit analysis in accordance with Standard Methods (1976).

The following species were run simultaneously in this 96-hour test: Leptasterias hexactis, Pagurus granosimanus, P. samuelis, Patiria miniata, Petrolisthes cinctipes, Pisaster brevispinus, and P. ochraceus.

2.10.20.2 RESULTS

Cumulative means and standard deviations of temperature data for each observation period are presented in TABLE 2.10.12-1.

Mortality occurred in the 26, 27 and 28C tanks. Plots of the mortality percentage versus time for each of these treatments are presented in FIGURE 2.10.20-1. Of the presumed dead animals, one, from the 28C tank, recovered after being placed in the ambient temperature recovery tank. Because it is not known which of the animals recorded as presumed dead in the test tank recovered, the mortality data were not altered to reflect this fact. All other presumed dead animals did not recover. Following the 96-hour test period, one "latent" mortality occurred among the animals from the 26C tank.

Probit regression lines were fitted by eye to the mortality data to determine the temperature which would cause 50 percent mortality at each observation period (FIGURE 2.10.20-2). The 96-hour ET50 value was determined to be approximately 26.0C. The estimators of the ET50 value thus calculated for each observation period were used to generate a curve of time to 50 percent mortality versus temperature, which is presented in FIGURE 2.10.20-3. The "incipient lethal" temperature, indicated by the vertical alignment of points to the left of the curve, was defined by the experiment to be 26.0C, which is equivalent to the 96-hour ET50 value.

2.10.20.3 DISCUSSION

Based on the results of this experiment, seawater temperatures in excess of 26.0C (79F) for four days would be expected to cause at least 50 percent mortality in a population of Pisaster giganteus. Little or no thermal mortality would be expected for exposures to temperatures at or below 25C (77F) for the same period of time. Exposure to 28C (82F) for 20 hours and to 27C (81F) for 44 hours would be expected to cause 100 percent mortality in a population of P. giganteus.

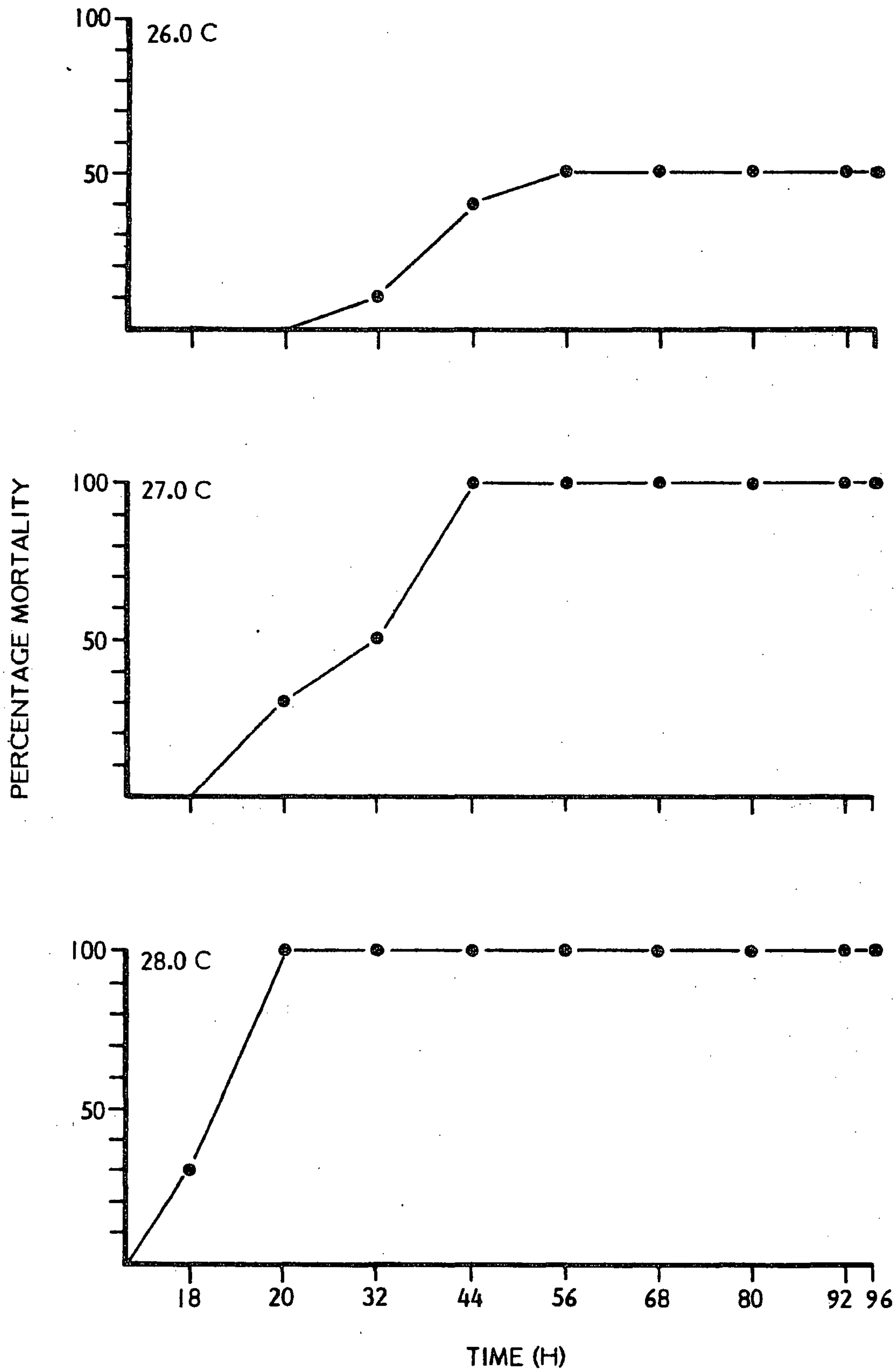

FIGURE 2.10.20-1

CUMULATIVE MORTALITY VERSUS TIME:
PISASTER GIGANTEUS HEAT TOLERANCE

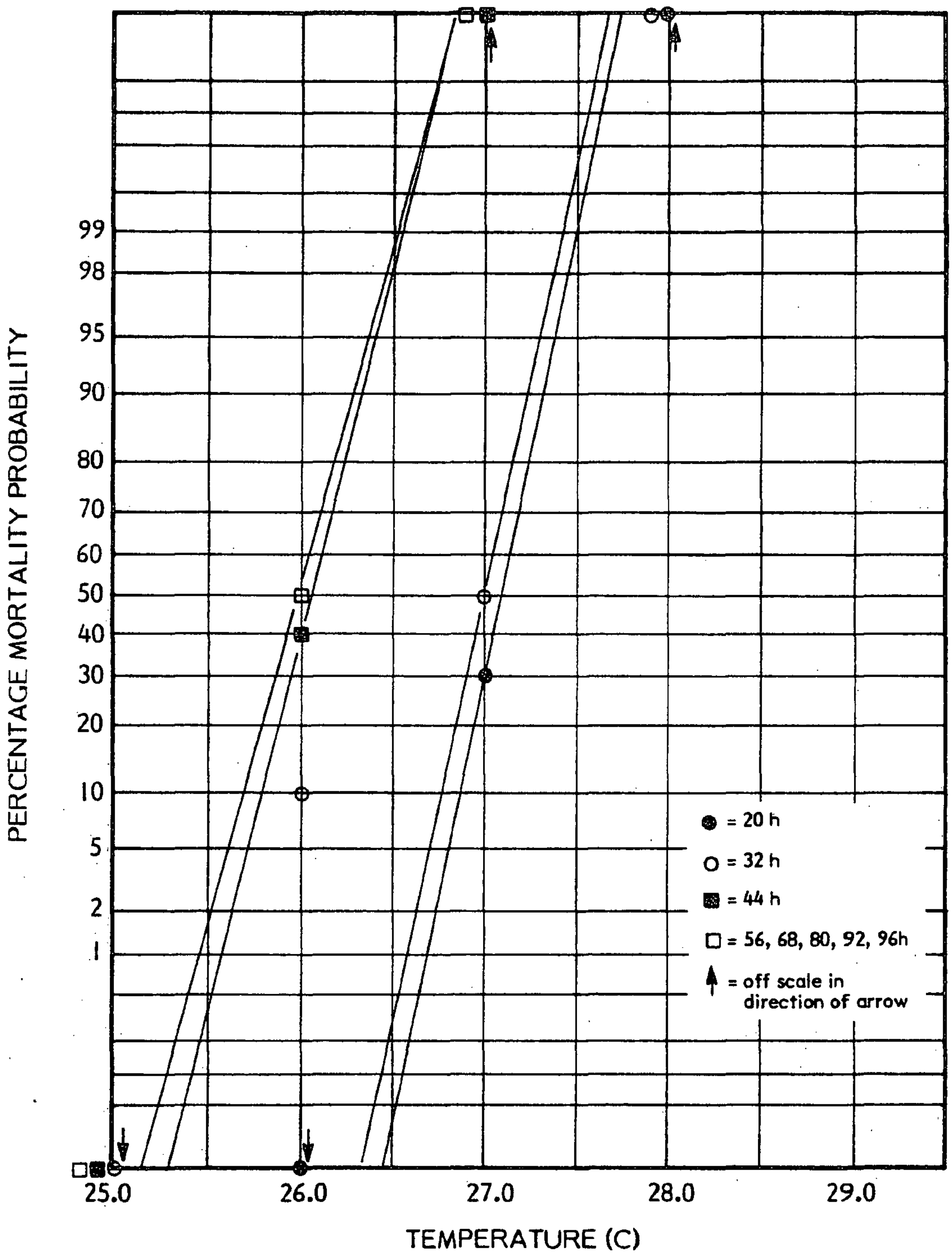

FIGURE 2.10.20-2

PERCENTAGE MORTALITY PROBABILITY VERSUS TEMPERATURE:
<u>PISASTER GIGANTEUS</u> HEAT TOLERANCE

The lines were fitted by eye.

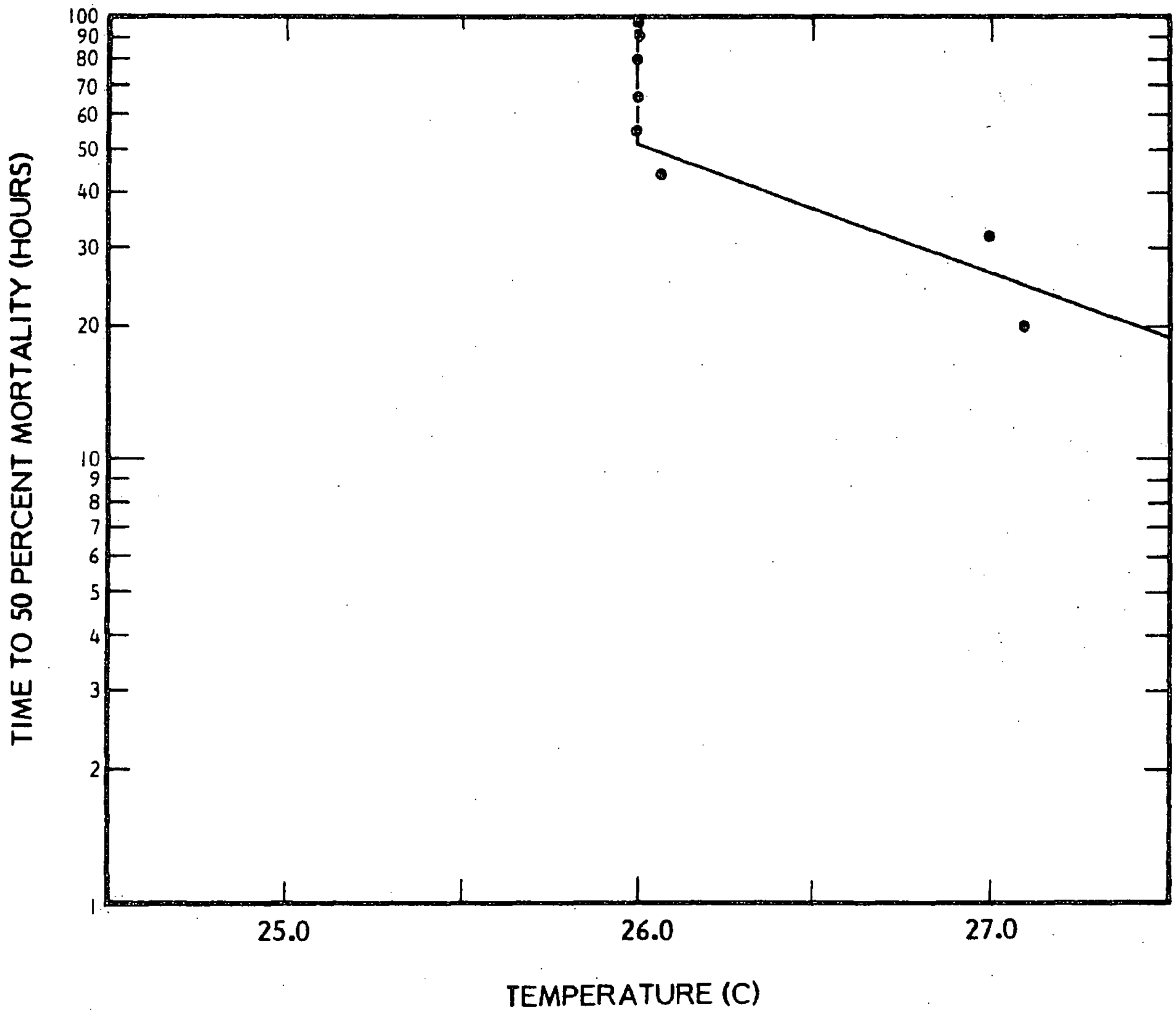

FIGURE 2.10.20-3

TIME TO 50 PERCENT MORTALITY VERSUS TEMPERATURE:
PISASTER GIGANTEUS HEAT TOLERANCE

2.10.21 PISASTER OCHRACEUS 96-HOUR MEDIAN EFFECTIVE TEMPERATURE (96 H ET50)

According to Morris et al. (1980), Pisaster ochraceus, the ochre sea star, occurs commonly on exposed rocky shores in the middle and lower intertidal zone. The species ranges into the subtidal to 88 m (288 ft) and geographically from Alaska to Point Sal, Santa Barbara County, California.

A review of the literature on P. ochraceus, which is an RIS for the Diablo Canyon 316(a) demonstration, was presented by PGandE (1979b).

2.10.21.1 METHODS

A summary of the methods employed in the heat tolerance experiment on Pisaster ochraceus was presented in SECTION 2.4.2. A more detailed account of the experimental procedure used in 96-hour ET50 experiments is presented in APPENDIX B (Procedure 319). The protocol for this experiment is included in APPENDIX C (experiment 19b).

The adult specimens of P. ochraceus used in this experiment were collected from April to July 1979 in the vicinity of Diablo Cove. The animals were held in the laboratory at ambient temperatures and were kept continuously submerged throughout their stay in the laboratory. During the holding period, the animals were carefully observed and those which appeared damaged or otherwise unhealthy were excluded from use in the experiment.

The experiment was carried out in 4 x 8 x 2 ft tanks, each of which (except the ambient tank) was programmed to a test temperature. Large specimens of P. ochraceus were held in the tanks inside 2 x 3 ft x 18 in. cages fabricated of 1/8 in. mesh Vexar screen attached to ½ in. PVC pipe frame with a fiberglass sheet bottom. The animals were continuously submerged during the course of the experiment. Temperatures were controlled and recorded by an Autodata Nine computer. The temperature data were transcribed from paper tapes onto data sheets and summarized as cumulative means and standard deviations for each observation period.

Observations were made after 8 hours and subsequently every 12 hours for the remainder of the 96-hour test period. Experimental target temperatures were 22, 23, 24, 25, 26, 27 and 28C. An ambient control treatment was also maintained. The number of animals tested was 10 each for the ambient and test tanks.

The criteria for death were the lack of ability to hold onto a surface and/or the absence of tube feet movement. Animals that were presumed dead were returned to an ambient temperature tank to assess possible recovery. Animals remaining alive at the end of the 96-hour test period were returned to ambient temperature. After about 72 hours (167 hours after the start of the 96-hour test) the animals were observed to determine if any "latent" mortality occurred. Mortality data were analyzed with probit analysis in accordance with Standard Methods (1976).

The following species were run simultaneously in this 96-hour test: Leptasterias hexactis, Pagurus granosimanus, P. samuelis, Patiria miniata, Petrolisthes cinctipes, Pisaster brevispinus, and P. giganteus.

2.10.21.2 RESULTS

Cumulative means and standard deviations of temperature data for each observation period are presented in TABLE 2.10.12-1.

Mortality occured in the 26, 27 and 28C tanks. Plots of the mortality percentage versus time for each of these treatments are presented in FIGURE 2.10.21-1. None of the presumed dead animals recovered. Following the 96-hour test period, one "latent" mortality occurred among the animals from the 26C tank.

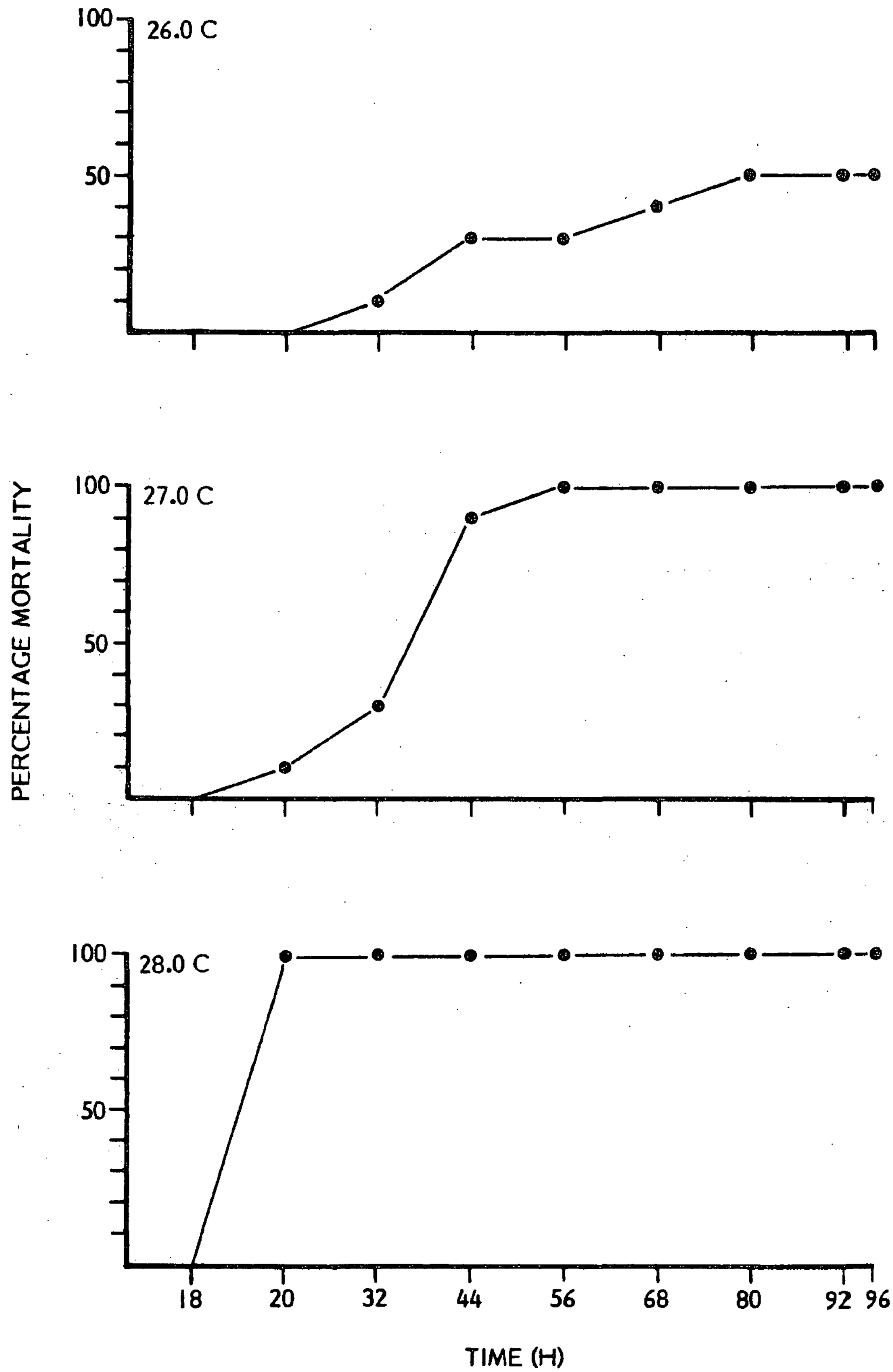

FIGURE 2.10.21-1

CUMULATIVE MORTALITY VERSUS TIME:
PISASTER OCHRACEUS HEAT TOLERANCE

Probit regression lines were fitted by eye to the mortality data to determine the temperature which would cause 50 percent mortality at each observation period (FIGURE 2.10.21-2). The 96-hour ET50 value was determined to be 26.0C. The estimators of the ET50 value thus calculated for each observation period were used to generate a curve of time to 50 percent mortality versus temperature, which is presented in FIGURE 2.10.21-3. The "incipient lethal" temperature, indicated by the vertical alignment of points to the left of the curve, was defined by the experiment to be 26.0C, which is equivalent to the 96-hour ET50 value.

2.10.21.3 DISCUSSION

Based on the results of this experiment, seawater temperatures in excess of 26C (79F) for four days would be expected to cause at least 50 percent mortality in a population of *Pisaster ochraceus*. Little or no thermal mortality would be expected for exposures to temperatures at or below 25C (77F) for the same period of time. Exposure to 27C (81F) for 56 hours and to 28C (82F) for 20 hours would be expected to cause 100 percent mortality in a population of *P. ochraceus*. However, it should be noted that *P. ochraceus* occurs most abundantly in the Diablo Canyon region in the intertidal zone, alternately submerged and exposed to the air, whereas in this experiment thermal exposure occurred during continual submergence. The environmental regime to which *P. ochraceus* is exposed in nature could have an ameliorative effect on the thermal stress responses obtained in this experiment.

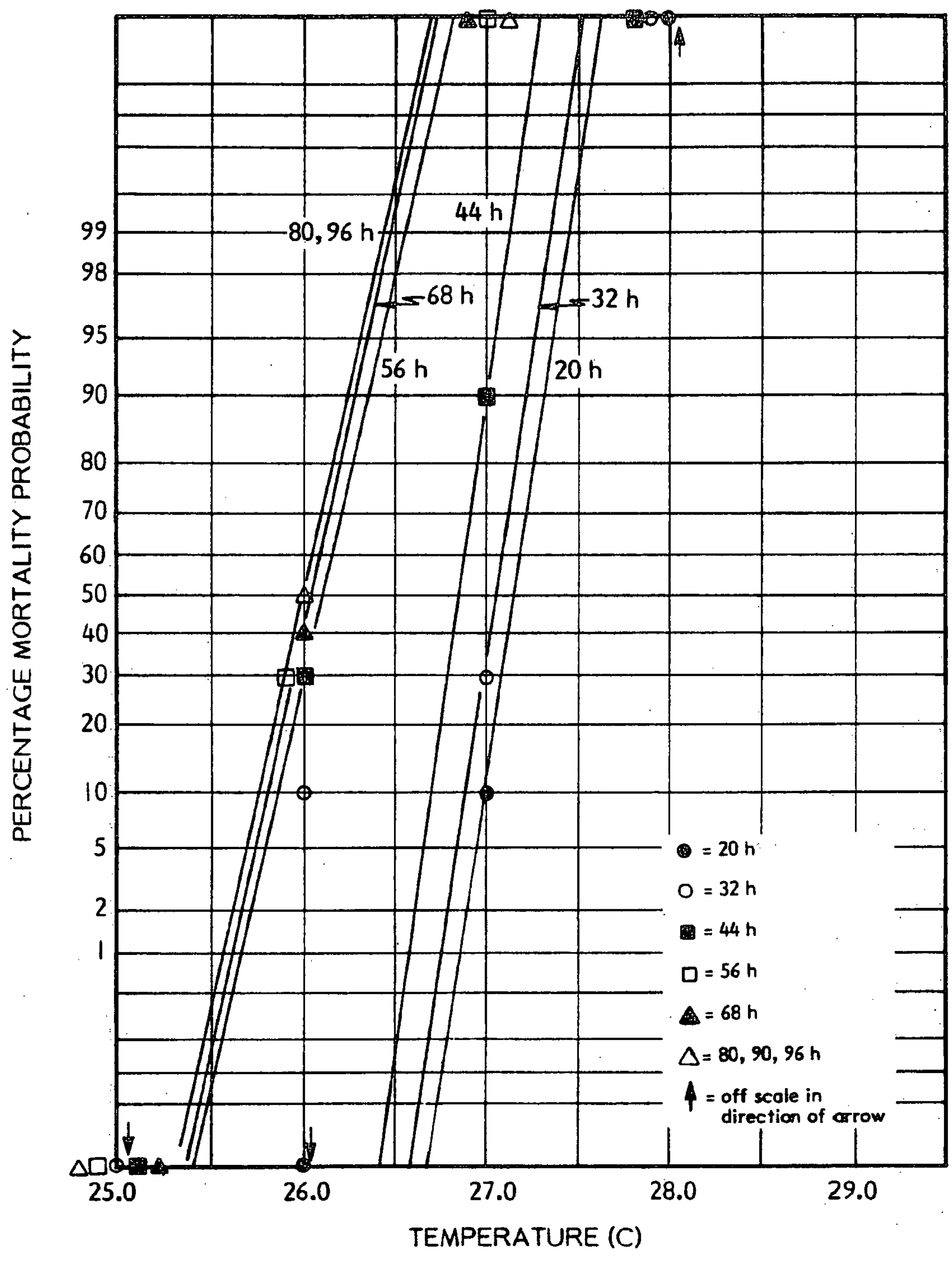

FIGURE 2.10.21-2

PERCENTAGE MORTALITY PROBABILITY VERSUS TEMPERATURE:
PISASTER OCHRACEUS HEAT TOLERANCE

The lines were fitted by eye.

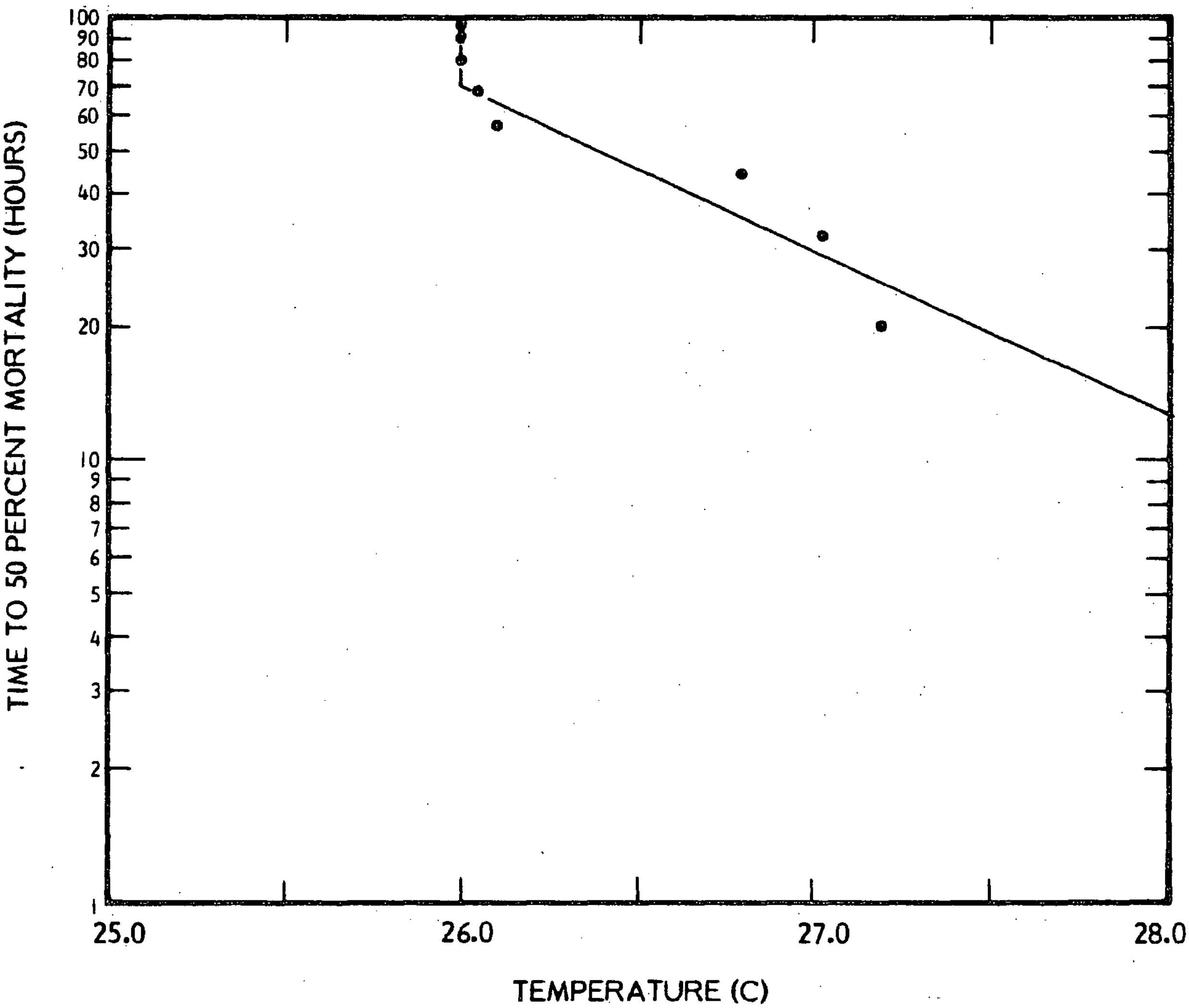

FIGURE 2.10.21-3

TIME TO 50 PERCENT MORTALITY VERSUS TEMPERATURE:
PISASTER OCHRACEUS HEAT TOLERANCE

2.10.22 PUGETTIA PRODUCTA 96-HOUR MEDIAN EFFECTIVE TEMPERATURE (96 H ET50)

This section reports the results of a heat tolerance experiment on the kelp crab, Pugettia producta (a Representative Important Species). A review of the literature pertaining to this species was presented in PGandE (1979b). The objective of this study was to determine the 96-hour ET50 of this species as an index to its thermal tolerance, and to provide some indication of the possible effects of the discharge on populations of kelp crabs in Diablo Cove.

2.10.22.1 METHODS

A summary of the methods employed in the heat tolerance experiment on this species was presented in SECTION 2.4.2. A more detailed description of the experimental procedure utilized in 96-hour ET50 experiments on invertebrates is presented in APPENDIX B (Procedure 319). The protocol for this experiment is included in APPENDIX C (experiment 19c).

Specimens tested included both juveniles and adults that were collected by hand at low tide. Specimens were acclimated to laboratory conditions at ambient temperature prior to testing. During the experiment crabs were kept completely submerged in test tanks which were subdivided to accommodate several species per test. Temperature was controlled and recorded by an Autodata Nine computer. Temperature data were transcribed and summarized as cumulative means and standard deviations for each observation period.

Observations were made after six hours and subsequently every 12 hours for the 96-hour test period. Experimental target temperatures were 19, 20.5, 22, 23.5, 25, 26.5, and 28 C. An ambient control (mean of 12.5 C) was also run. Test populations consisted of ten animals per treatment.

The criterion for death was the loss of ability to walk when gently prodded. Animals that were presumed dead were returned to an ambient temperature tank to assess possible recovery. Mortality data were analyzed with probit analysis in accordance with Standard Methods (1976).

2.10.22.2 RESULTS

Cumulative means and standard deviations of temperature data for each observation period are presented in TABLE 2.6-1.

During the 96-hour test period, 10 percent mortality occurred in the 20.5, 22, 23.5, and 25 C tanks. At 28 C, 80 percent mortality had occurred at 42 hours, and 100 percent of the test organisms had died after 78 hours (FIGURE 2.10.22-1). None of the presumed dead animals recovered after being held for 162 hours at ambient temperature. Because so few intermediate mortalities were obtained, further analysis does not seem warranted. The results indicate that the 96-hour ET50 value for this species lies between 26.5 and 28 C.

2.10.22.3 DISCUSSION

Using the criterion of inability to walk when prodded to determine death, the results of this experiment suggest that exposure to seawater temperatures of 28 C (82.4 F) for a period of four days would cause 100 percent mortality in a population of the kelp crab, Pugettia producta. Exposure to temperatures of 26.5 C (79.7 F) or less for the same period of time would not cause appreciable mortalities. In the only known published account relating to the heat tolerance of this species, North and Schaeffer (1964) found that the upper lethal temperature of kelp crabs subjected to 1-hour heat spikes was 32.5 C (90.5 F). Thus, there is evidence to suggest that Pugettia producta is a fairly heat tolerant species, in regard to both short- and long-term exposures to elevated temperatures.

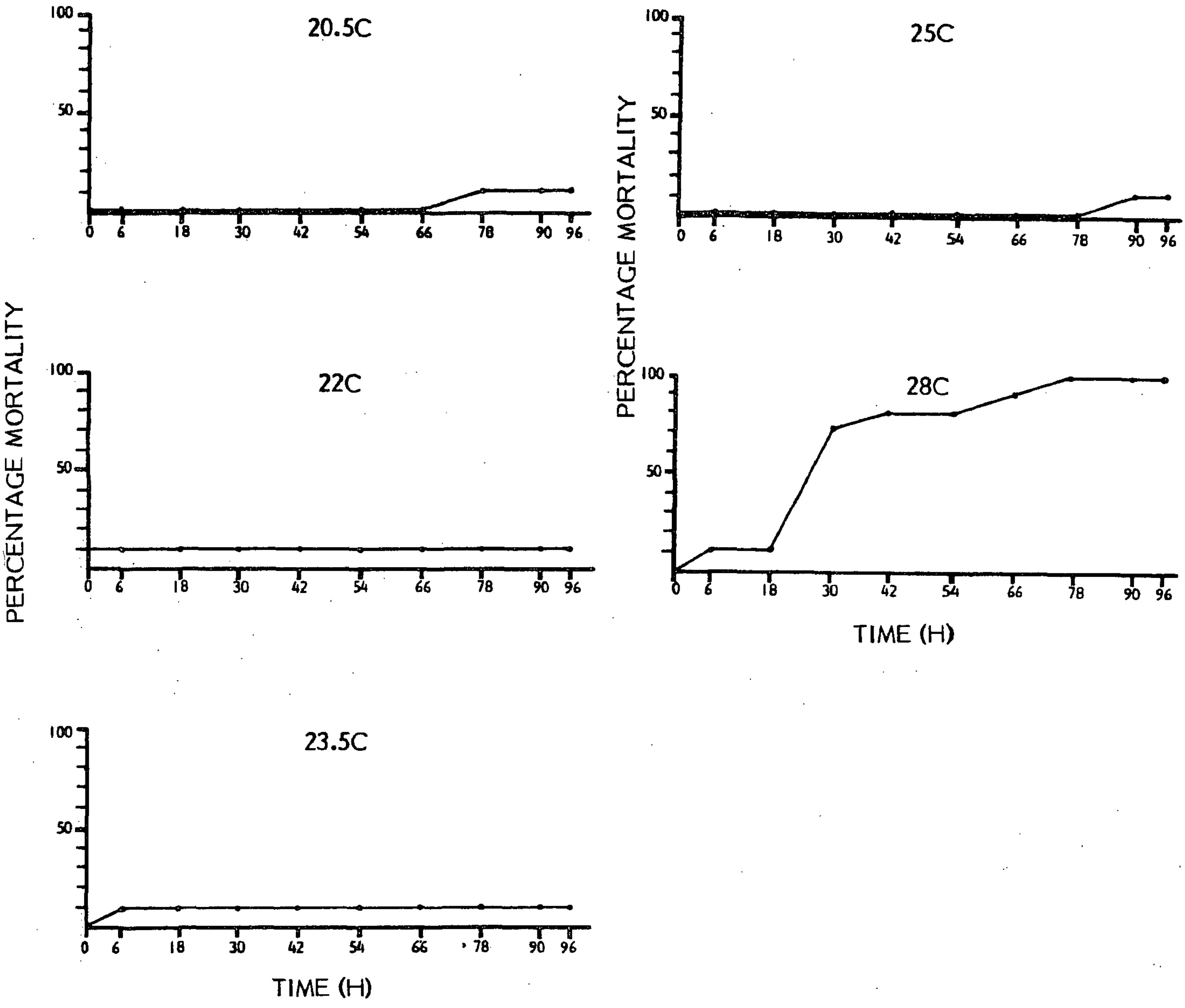

FIGURE 2.10.22-1

<u>PUGETTIA</u> <u>PRODUCTA</u> HEAT TOLERANCE

2.10.23 PUGETTIA RICHII 96-HOUR MEDIAN EFFECTIVE TEMPERATURE (96 H ET50)

The decorator crab, Pugettia richii, while not an RIS species, is a congener of the kelp crab, P. producta. P. richii occupies a similar habitat in the Diablo Canyon study area and was simultaneously tested in heat tolerance experiments with the RIS species, Pugettia producta and Cancer antennarius. A search of the literature on this species found no published accounts relating to its heat tolerance. The purpose of this experiment was, therefore, to determine the 96-hour ET50 as an index to the thermal tolerance of this species.

2.10.23.1 METHODS

A summary of the methods employed in the heat tolerance experiment on this species was presented in SECTION 2.4.2. A more detailed description of the experimental procedure utilized in 96-hour ET50 experiments on invertebrates presented in APPENDIX B (Procedure 319). The protocol for this experiment is included in APPENDIX C (experiment 19c).

Specimens tested included both juveniles and adults that were collected by hand at low tide. Specimens were acclimated to laboratory conditions at ambient temperature prior to testing. During the experiment crabs were kept completely submerged in test tanks which were subdivided to accommodate several species per test. Temperature was controlled and recorded by an Autodata Nine computer. Temperature data were transcribed and summarized as cumulative means and standard deviations for each observation period.

Observations were made after six hours and subsequently every 12 hours for the 96-hour test period. Experimental target temperatures were 19, 20.5, 22, 23.5, 25, 26.5, and 28 C. An ambient control (mean of 12.5 C) was also run. Test populations consisted of ten animals per treatment.

The criterion for death was the loss of ability to walk when gently prodded. Animals that were presumed dead were returned to an ambient temperature tank to assess possible recovery.

2.10.23.2 RESULTS

TABLE 2.6-1 presents cumulative means and standard deviations for each observation period.

Mortalities occurred at each test temperature by the end of the 96-hour test period. In addition, 20 percent of the animals in the ambient temperature control had also died after 96-hours (FIGURE 2.10.23-1). After 96 hours, 20 percent of the animals at 25 C had died, while 100 percent of the test groups at 26.5 and 28 C had died. None of the presumed dead animals recovered after being placed in the ambient temperature recovery tank. Because of the distribution of mortalities below 26.5 C and the occurrence of 20 percent mortality in the control, detailed probit analysis was not conducted. It is apparent from the results, however, that the 96-hour ET50 value for this species is between 25 and 26.5 C.

2.10.23.3 DISCUSSION

The results of this experiment indicate that seawater temperatures of 26.5 C (79.7 F) would cause 100 percent mortality in a population of Pugettia richii in a very short period of time, probably six hours or less. Exposure to temperatures below 25 C (77 F) for much longer periods would probably not cause appreciable mortality. The heat tolerance of this species appears to be lower than that of the related kelp crab, Pugettia producta, which suffered no mortalities at 26.5 C (79.7 F) during a 96-hour exposure.

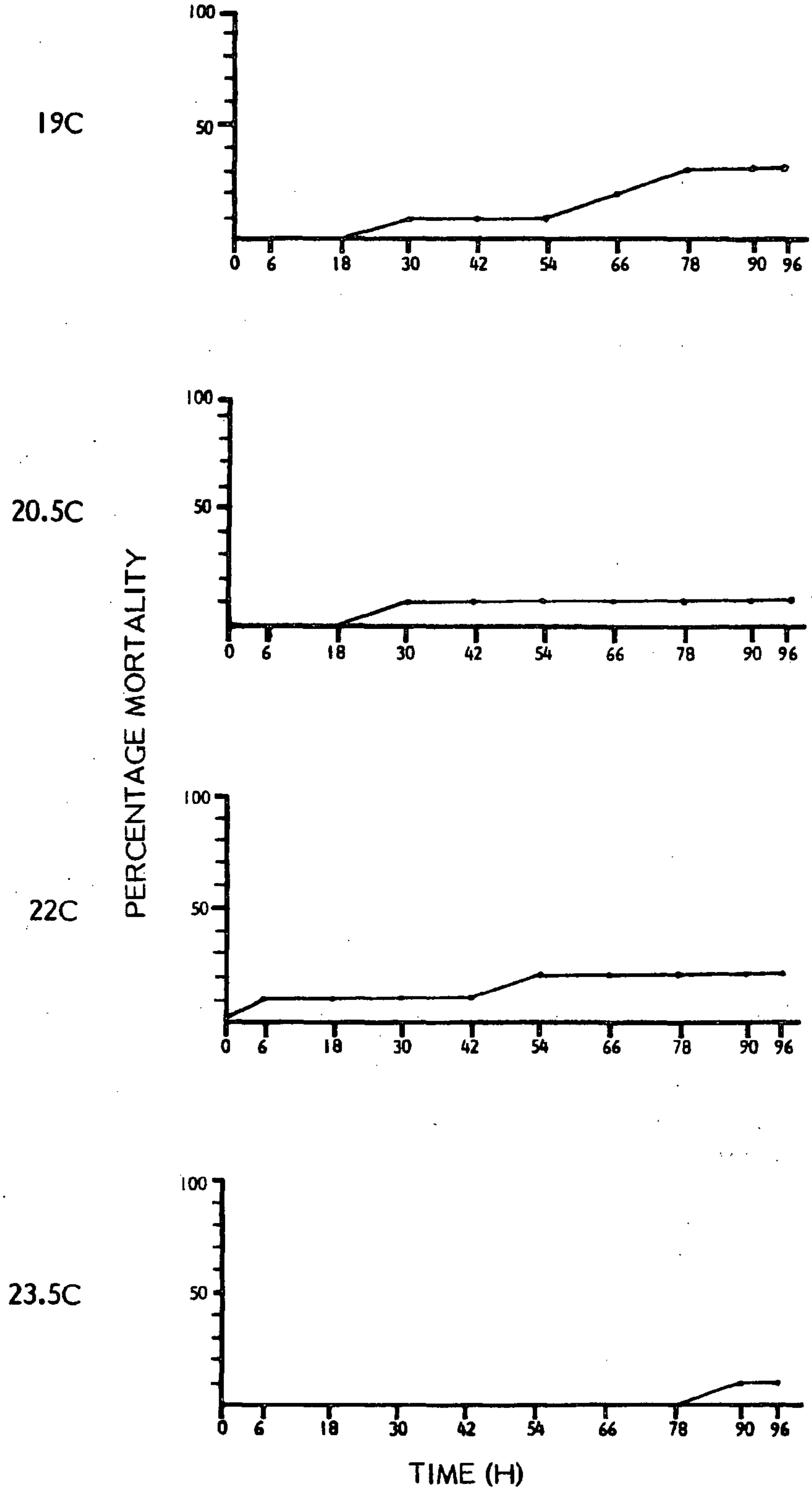

FIGURE 2.10.23-1

CUMULATIVE MORTALITY VERSUS TIME:
PUGETTIA RICHII HEAT TOLERANCE

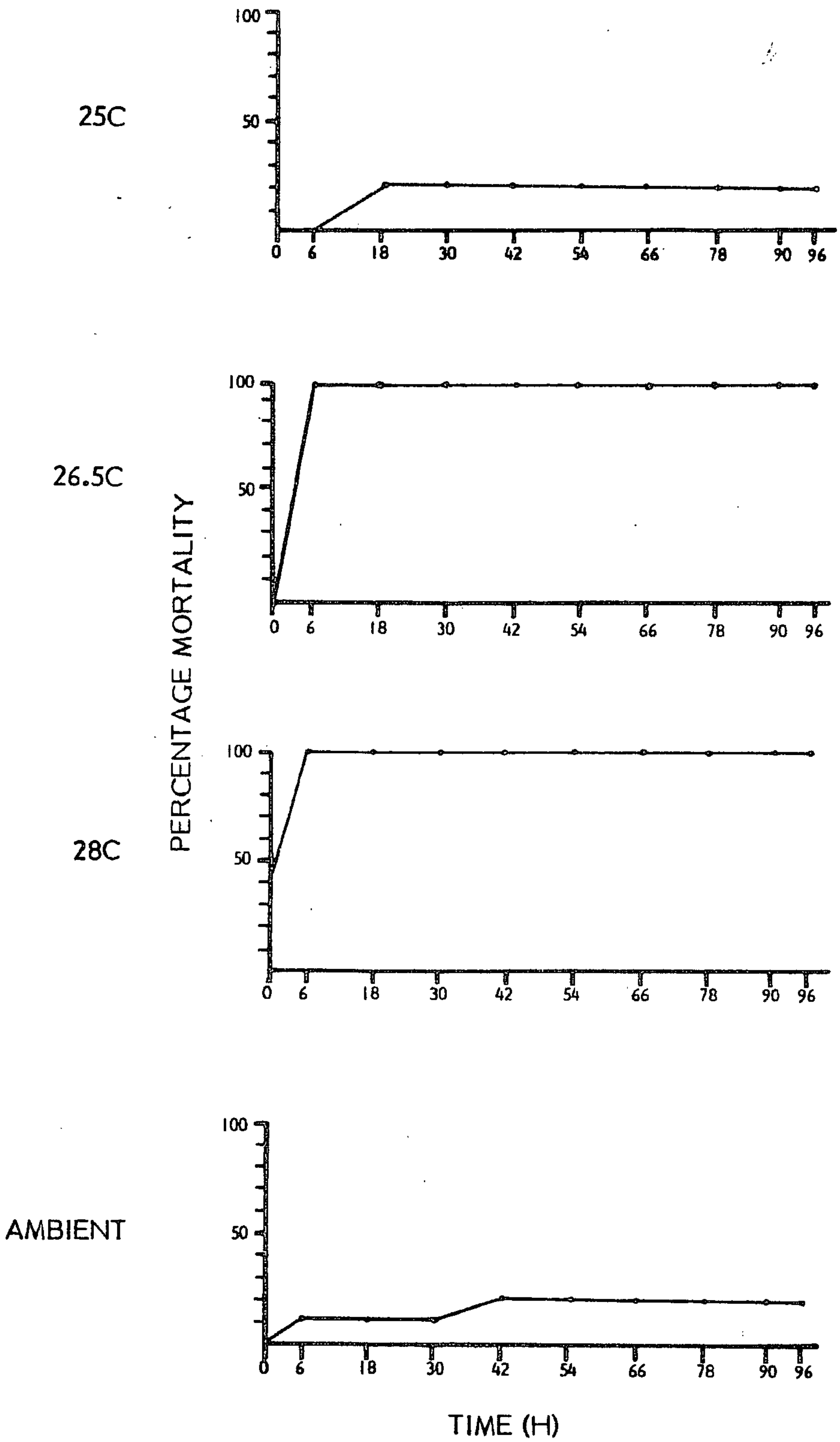

FIGURE 2.10.23-1 (CONT.)

2.10.24 STRONGYLOCENTROTUS PURPURATUS 96-HOUR MEDIAN EFFECTIVE TEMPERATURE (96 H ET50)

According to Morris et al. (1980), the purple sea urchin, Strongylocentrotus purpuratus, occurs commonly in the lower intertidal zone on rocky shores and pilings, especially in areas of moderate to strong wave action, and in the subtidal to depths of 160 m (525 ft). Its geographical distribution extends from Vancouver Island, British Columbia, Canada to Isla Cedros, Baja California, Mexico. It is one of the best-known sea urchins in the world and the subject of much research.

Strongylocentrotus purpuratus is an RIS but has been recorded in the 316(a) samples as an abundant species only at subtidal station 20-20 (PGandE 1979a). A review of the literature on this species was completed by PGandE (1979b). With respect to thermal tolerance, Farmanfarmaian and Giese (1963) reported that S. purpuratus has a sharp upper tolerance limit because no animal survived a four-day exposure to 25C, whereas animals exposed to 23.5C appeared normal. This experiment was carried out to confirm their results.

2.10.24.1 METHODS

A summary of the methods employed in the heat tolerance experiment on Strongylocentrotus purpuratus was presented in SECTION 2.4.2. A more detailed account of the experimental procedure used in 96-hour ET50 experiments is presented in APPENDIX B (Procedure 319). The protocol for this experiment is included in APPENDIX C (experiment 19c).

The adult specimens of S. purpuratus used in this experiment were collected on 28 June 1979 at "Postelsia Point" at the north end of Diablo Cove. The animals were held in the laboratory at ambient temperatures and were kept continuously submerged throughout their stay in the laboratory. During the holding period, the animals were carefully observed and those which appeared damaged or otherwise unhealthy were excluded from use in the experiment.

The animals were separated from other species run simultaneously in the 4 x 8 ft test and control tanks by being placed within 12 x 18 in. compartments constructed of 1/8 in. Vexar screen. Temperatures were controlled and recorded by an Autodata Nine computer. The temperature data were transcribed from paper tapes onto data sheets and summarized as cumulative means and standard deviations for each observation period.

Observations were made after 6 hours and subsequently every 12 hours for the remainder of the 96-hour test period. Experimental target temperatures were 19, 20.5, 22, 23.5, 25, 26.5 and 28C. An ambient control treatment was also maintained.

The criteria for death were the failure to hold onto a substrate when gently prodded and lack of movement of tube feet. Animals that were presumed dead were returned to an ambient temperature tank to assess possible recovery. Mortality data were analyzed with probit analysis in accordance with Standard Methods (1976).

The following species were run simultaneously in this 96-hour test: Acanthina spirata, Cancer antennarius, Collisella digitalis, C. limatula, C. pelta, C. scabra, Hemigrapsus nudus, Pachygrapsus crassipes, Pugettia producta, and P. richii.

2.10.24.2 RESULTS

Cumulative means and standard deviations of temperature data for each observation period are presented in TABLE 2.10.4-1.

Mortality occurred in the 23.5, 25.0, 26.5 and 28.0C tanks. The mortality percentage data versus time are plotted in FIGURE 2.10.24-1. None of the presumed dead animals recovered after being placed in the ambient temperature recovery tank.

Probit regression lines were fitted by eye to the mortality data to determine the temperature which would cause 50 percent mortality at the 6- and 18-hour

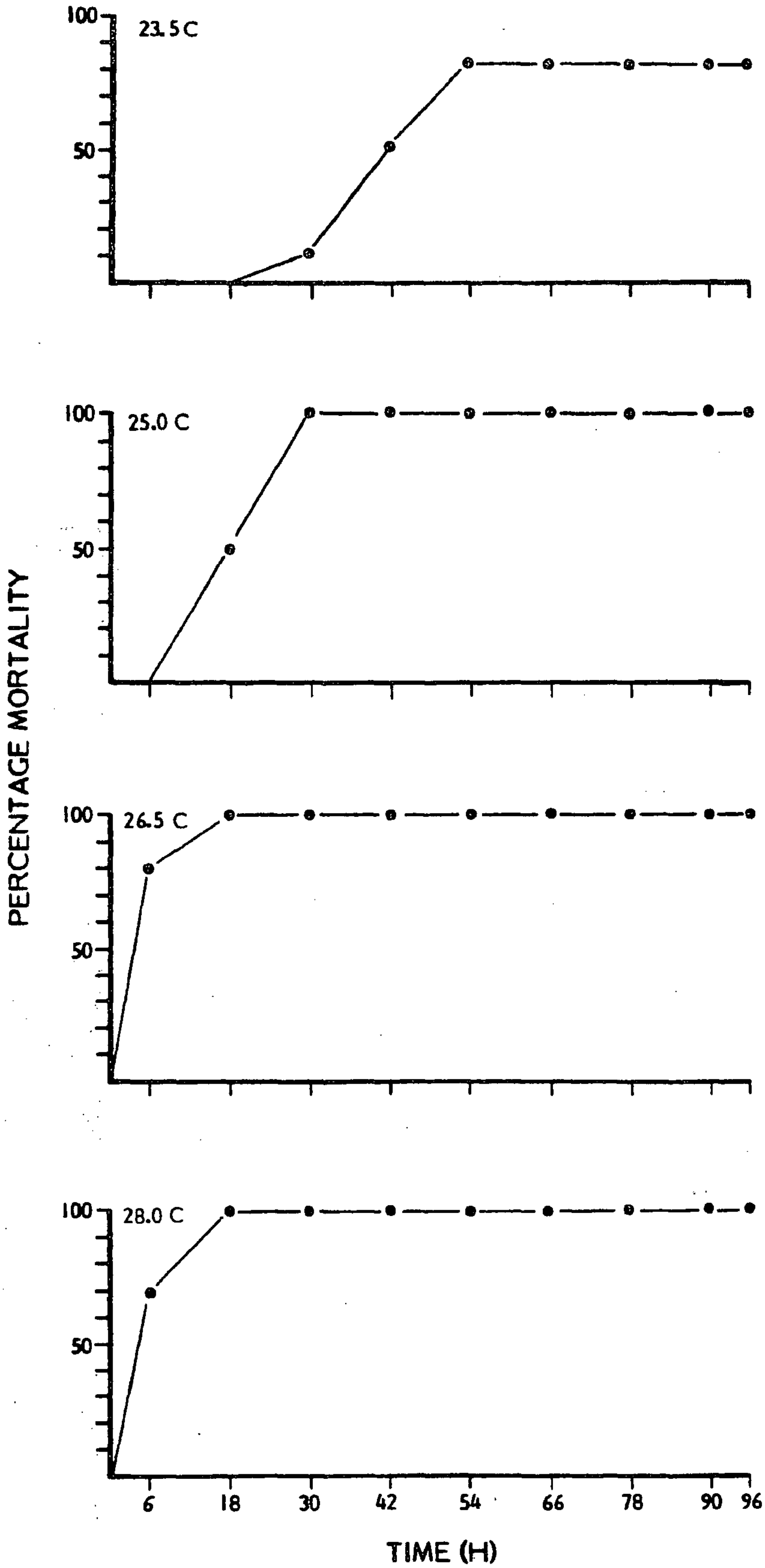

FIGURE 2.10.24-1

CUMULATIVE MORTALITY VERSUS TIME:
STRONGYLOCENTROTUS PURPURATUS HEAT TOLERANCE

observation periods (FIGURE 2.10.24-2). The 96-hour ET50 value could not be determined from the data but occurs in the range 22.0-23.5C (0 and 100 percent mortality, respectively, at 96 hours).

2.10.24.3 DISCUSSION

Based on the results of this experiment, seawater temperatures at or above 25.0C (77.0F) for four days would be expected to cause 100 percent mortality in a population of Strongylocentrotus purpuratus. No thermal mortality would be expected for exposures to temperatures below 22.0C (71.6F) for the same period of time.

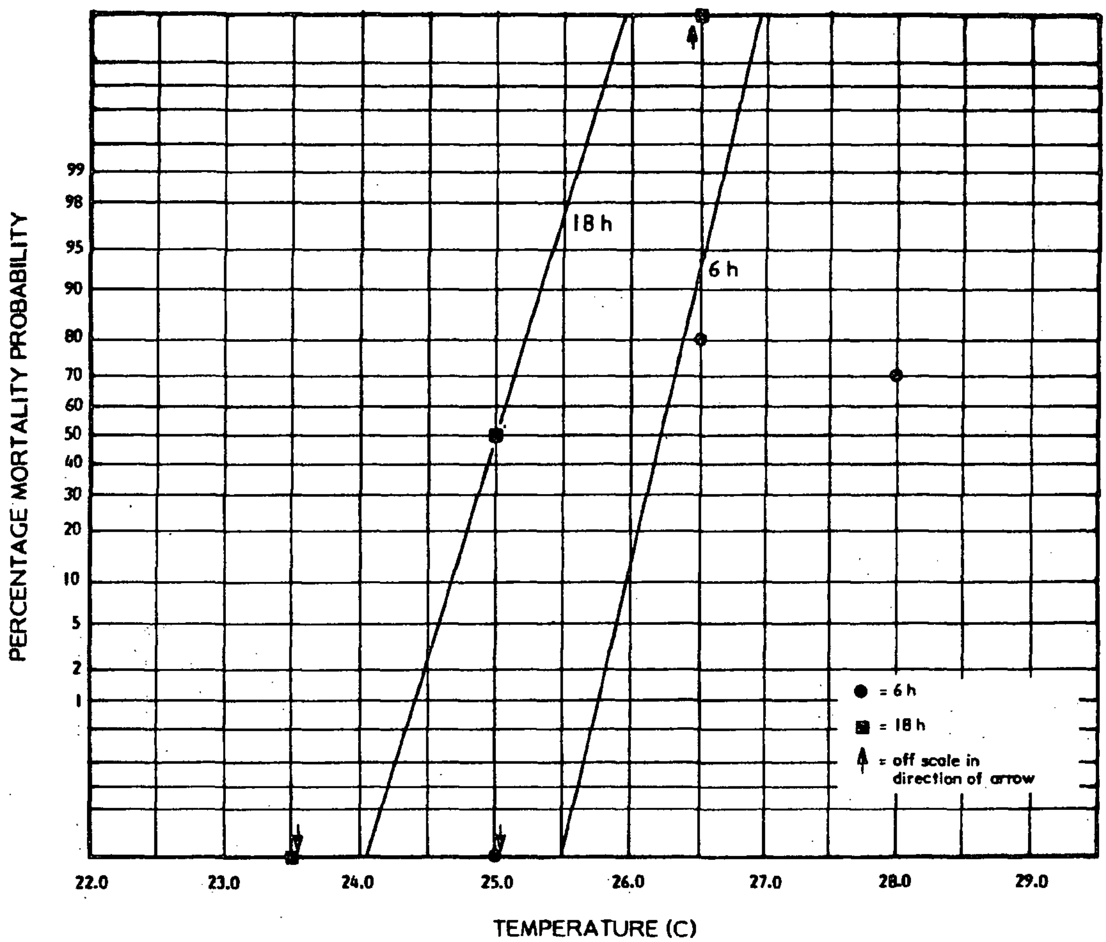

FIGURE 2.10.24-2

PERCENTAGE MORTALITY PROBABILITY VERSUS TEMPERATURE: STRONGYLOCENTROTUS PURPURATUS HEAT TOLERANCE

2.10.25 TEGULA BRUNNEA 96-HOUR MEDIAN EFFECTIVE TEMPERATURE (96 H ET50)

A review of the literature on the snail Tegula brunnea was presented in PGandE (1979b). The results of 96-hour tests to determine the median effective temperature (ET50), i.e., the temperature at which 50 percent of the test population survived, are reported on in this section. Two experiments were conducted on this species. In the first experiment, ambient acclimated animals were tested. In the second, animals that had been acclimated to high temperatures in a growth experiment were subsequently tested in a 96-hour ET50 experiment.

2.10.25.1 AMBIENT ACCLIMATION

The study objective of this experiment was to determine the 96-hour ET50 value for ambient acclimated Tegula brunnea populations in Diablo Cove as an index of their thermal tolerance. There are no published accounts of thermal tolerance studies of any type on this species. In addition, the time course of mortality was recorded for the 96-hour period. The purpose of this study was to provide information on the response of natural populations to elevated temperatures in Diablo Cove resulting from the power plant discharge.

Methods

A summary description of the laboratory methods employed in the investigations into heat tolerance of Tegula brunnea was presented in SECTION 2.4.2. For a more detailed description of the laboratory methods see APPENDIX B (Procedure 319) and protocol see APPENDIX C (experiment 19a). The individuals used in this experiment were originally collected in November and December 1978 from Macrocystis beds in the power plant intake cove and the northwest point of Diablo Cove.

During the experiment, the animals were placed in cages made of 1 ft lengths of 3 in. diameter PVC pipe with 1/8 in. mesh plastic screening at both ends and with an airstone fitted inside the pipe. The cages were kept continuously submerged in the laboratory test tanks. Temperature data were recorded hourly by an Autodata Nine computer, transcribed, and summarized as cumulative means and standard deviations at each observation period for each test tank.

Test animals were observed at 12-hour intervals during the 96-hour period at test temperatures of 15.1 C and ranging from 23.1 to 28.8 C. A control group of animals was maintained and observed at ambient temperatures.

Failure of an animal to retract into its shell after prodding constituted the criterion for "ecological death" (effective temperature, ET) during the experiment. Animals which met this criterion were returned to ambient temperatures to observe any recovery. Nine to ten animals were tested in each treatment group. Probit analysis of the mortality data was conducted in accordance with Standard Methods (1975).

Results

Cumulative means and standard deviations of the temperature data for all test tanks are given in TABLE 2.4-6 (see SECTION 2.4.2).

All "dead" animals were replaced into ambient seawater to determine if they would recover. At 162-hours, none of the dead animals had recovered. All live animals at the end of the 96-hour test period were placed into ambient seawater to determine whether any "latent" mortality would occur. No latent mortalities were observed at 162 hours.

No mortality was observed in the ambient (control), 15.1 or 23.1 C tanks. The cumulative mortalities for the tanks in which some mortality occurred are presented for all observation periods in FIGURE 2.10.25-1. Probit regression lines were fitted by eye to determine the temperature at 50 percent mortality at each observation period. Examples of these plots for the 40 and 96-hour observations are presented in FIGURE 2.10.25-2. The 96-hour ET50 value was 25.7 C. Estimations of the temperatures at which 50 percent mortality occurred

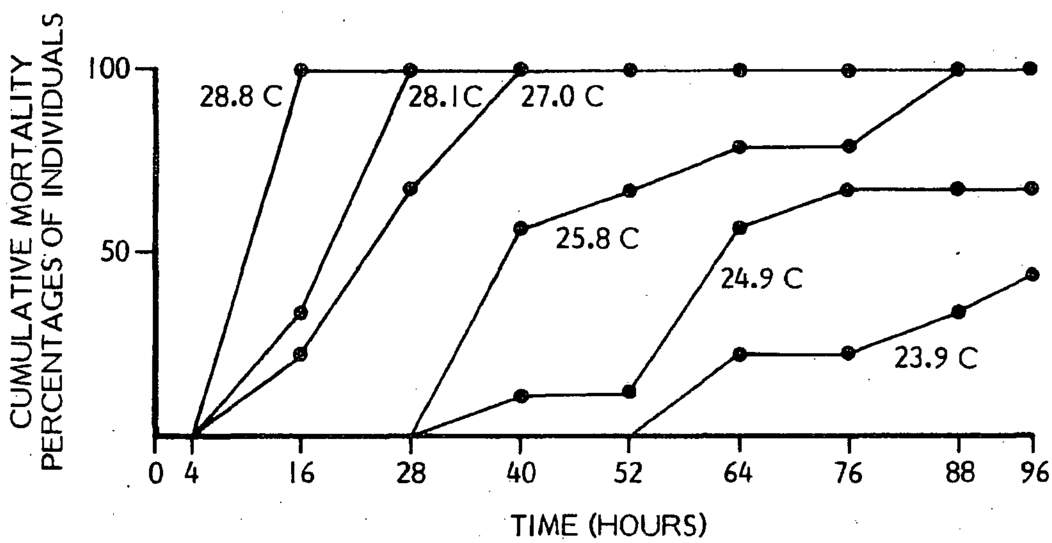

FIGURE 2.10.25-1

CUMULATIVE MORTALITY VERSUS TIME: TEGULA BRUNNEA HEAT TOLERANCE

The animals were acclimated to ambient temperatures and subjected to six test temperatures. Duplicate points at 100 percent mortality are not shown.

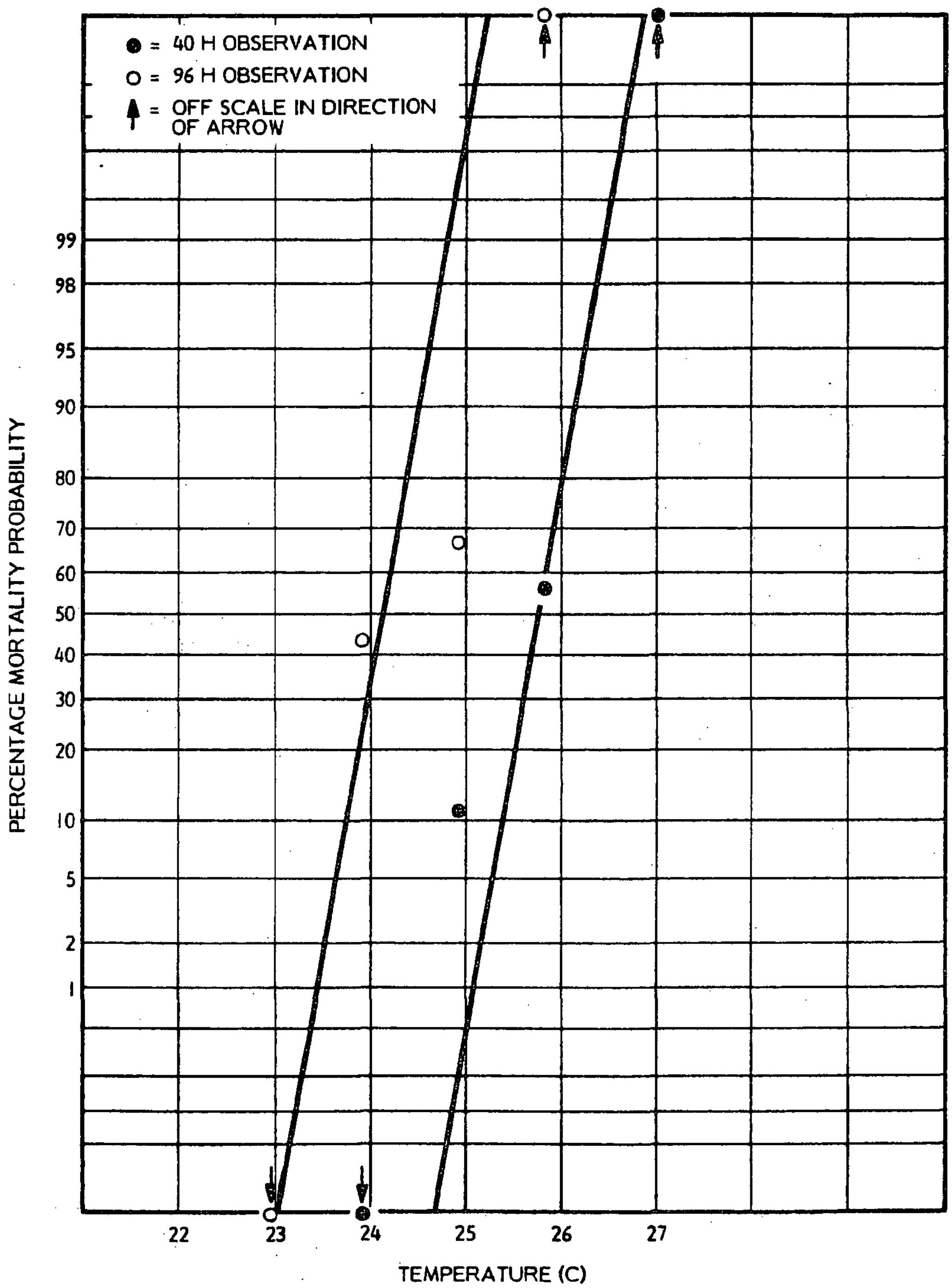

FIGURE 2.10.25-2

PERCENTAGE MORTALITY PROBABILITY
VERSUS TEMPERATURE AT 40 AND 96 HOURS:
TEGULA BRUNNEA HEAT TOLERANCE

The animals were acclimated to ambient temperatures.

at each observation period were derived from the probability plots and used to generate curves of time to 50 percent mortality versus temperature shown in FIGURE 2.10.25-3. The "incipient lethal temperature," which is generally indicated by a vertical alignment of points to the left, was not defined in this experiment.

Discussion

Using the criterion of lack of response to prodding to define death, seawater temperatures exceeding 25.7 C (78.3 F) would be expected to cause more than half of the exposed population of Tegula brunnea to die. Lower temperatures would produce fewer mortalities and no mortality would be expected at and below 23.1 C (73.6 F).

2.10.25.2 HIGH TEMPERATURE ACCLIMATION

During early 1979, a three-month growth experiment was conducted on T. brunnea during which groups of animals were acclimated to elevated temperatures. This 96-hour ET50 experiment was conducted following the growth experiment on some of the groups, to provide a comparative basis for evaluating possible thermal response differences between animals acclimated to various temperatures.

Methods

A summary of the methods employed in the heat tolerance experiment on Tegula brunnea was presented in SECTION 2.4.2. A more detailed account of the experimental procedure used in 96-hour ET50 experiments is presented in APPENDIX B (Procedure 319). The protocol for this experiment is included in APPENDIX C (experiment 19b).

The adult specimens of T. brunnea used in this experiment were collected on 13 December 1978 just south of Diablo Cove. The animals were used in a three-month growth experiment conducted in early 1979. From that experiment, three

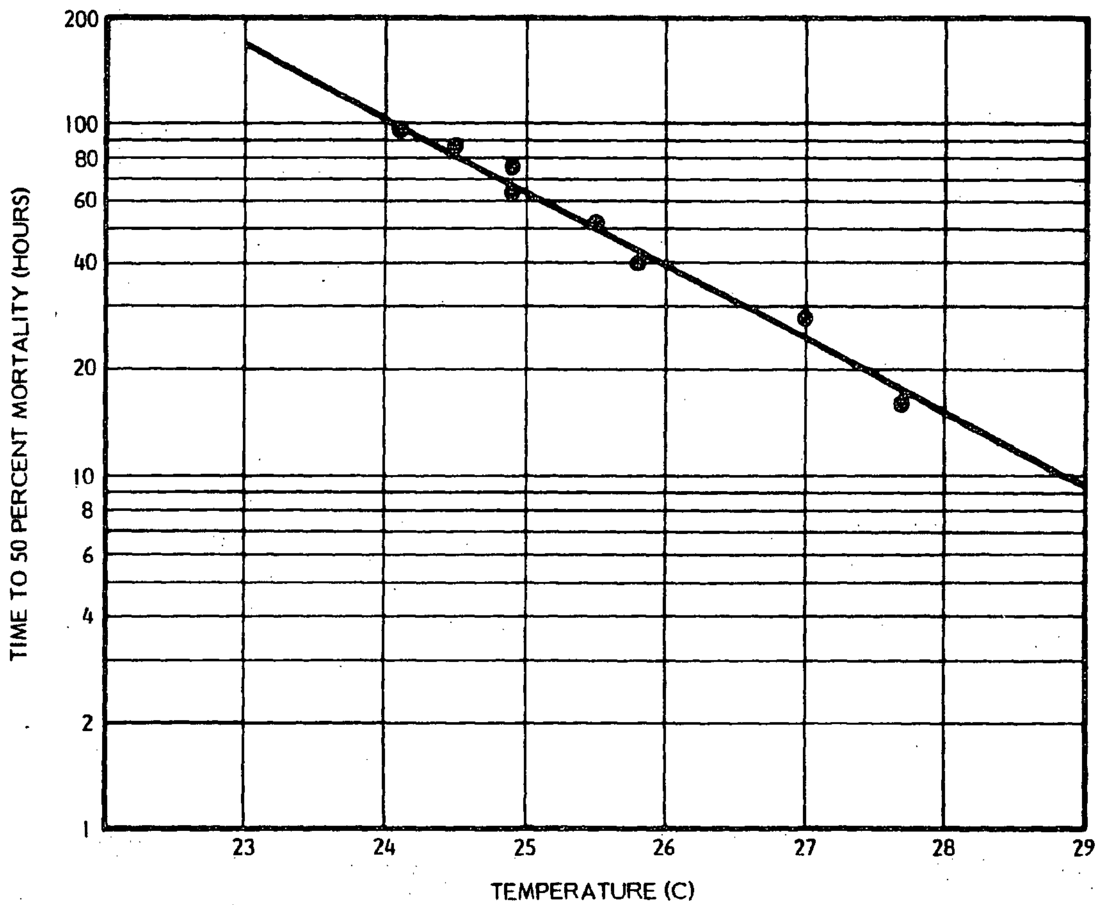

FIGURE 2.10.25-3

TIME TO 50 PERCENT MORTALITY VERSUS TEMPERATURE: TEGULA BRUNNEA HEAT TOLERANCE groups of animals, acclimated (for a period greater than 100 days) to 12, 15 and 24C were tested in this 96-hour ET50 experiment. The animals were kept continuously submerged throughout their stay in the laboratory.

The animals were kept in the test and control tanks underneath a slip-fit coupling for 3 in. PVC pipe screened at one end with 1/8 in. mesh Vexar plastic. These cages were separated from potential predators included in the same experiment by being placed within a 12 x 18 in. compartment made of 1/8 in. Vexar in a 4 x 8 x 2 ft tank. Temperatures were controlled and recorded by an Autodata Nine computer. The temperature data were transcribed from paper tapes onto data sheets and summarized as cumulative means and standard deviations for each observation period.

Observations were made after 8 hours and subsequently every 12 hours for the remainder of the 96-hour test period. Experimental target temperatures ranged from 22 to 28C, depending on the acclimation temperature of the animals. The control tanks approximated the acclimation temperature of each group: ambient (for the 12C group), 16C and 24C. The number of animals tested was nine or ten in each test and control condition.

The criterion for death was failure to respond (retract) when prodded. Animals that were presumed dead were returned to an ambient temperature tank to assess possible recovery. Animals remaining alive at the end of the 96-hour test period were returned to ambient temperature. After about 72 hours (167 hours after the start of the 96-hour test) the animals were observed to determine if any "latent" mortality occurred. Mortality data were analyzed with probit analysis in accordance with Standard Methods (1976).

The following species were run simultaneously in this 96-hour test: *Leptasterias hexactis*, *Pagurus granosimanus*, *P. samuelis*, *Patiria miniata*, *Petrolisthes cinctipes*, *Pisaster brevispinus*, *P. giganteus* and *P. ochraceus*.

Results

Cumulative means and standard deviations of temperature data for each observation period are presented in TABLE 2.10.12-1.

12C ACCLIMATED GROUP. Mortality occurred in the 25, 26, 27 and 28C tanks. Plots of the mortality percentage versus time for each of these treatments are presented in FIGURE 2.10.25-4. None of the presumed dead animals recovered after being placed in the ambient temperature recovery tank. Following the 96-hour test period, no "latent" mortalities occurred among the animals from the test tanks.

Probit regression lines were fitted by eye to the mortality data to determine the temperature which would cause 50 percent mortality at each observation period (FIGURE 2.10.25-5). The 96-hour ET50 value was determined to be 25.2C.

15C ACCLIMATED GROUP. Mortality occurred in the 25, 26, 27 and 28C tanks. Plots of the mortality percentage versus time for each of these treatments are presented in FIGURE 2.10.25-6. None of the presumed dead animals recovered after being placed in the 16C temperature recovery tank. Following the 96-hour test period, one "latent" mortality occurred among the animals from the 26C tank.

Probit regression lines were fitted by eye to the mortality data to determine the temperature which would cause 50 percent mortality at each observation period (FIGURE 2.10.25-7). The 96-hour ET50 value was determined to be 25.6C.

24C ACCLIMATED GROUP. Mortality occurred in the 26, 27 and 28C tanks. Plots of the mortality percentage versus time for each of these treatments are presented in FIGURE 2.10.25-8. None of the presumed dead animals recovered after being placed in the 24C temperature recovery tank. Following the 96-hour test period, one "latent" mortality occurred among the animals from the 26C tank and two occurred among the animals from the 27C tank.

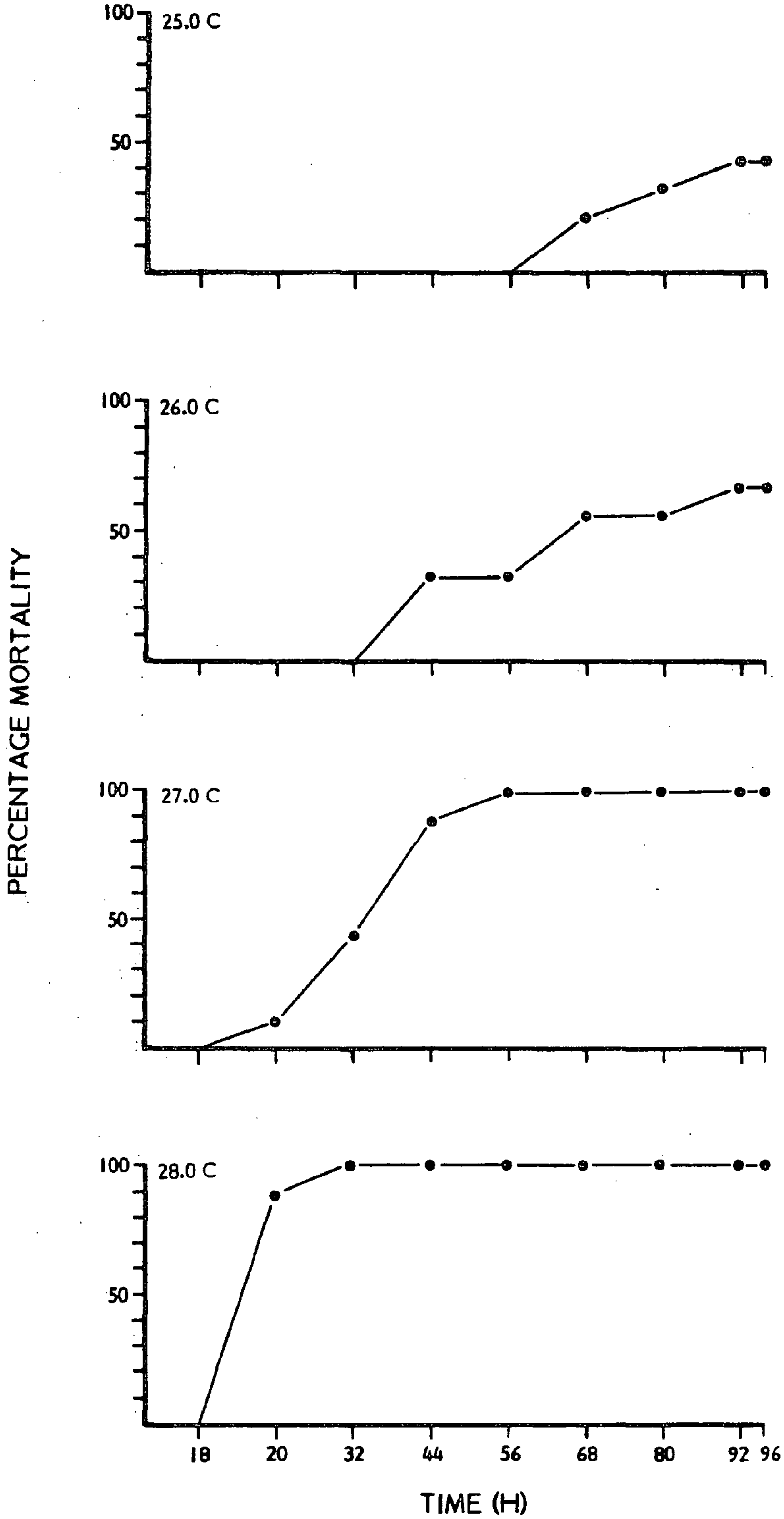

FIGURE 2.10.25-4

CUMULATIVE MORTALITY VERSUS TIME FOR 12 C ACCLIMATED ANIMALS: *TEGULA BRUNNEA* HEAT TOLERANCE

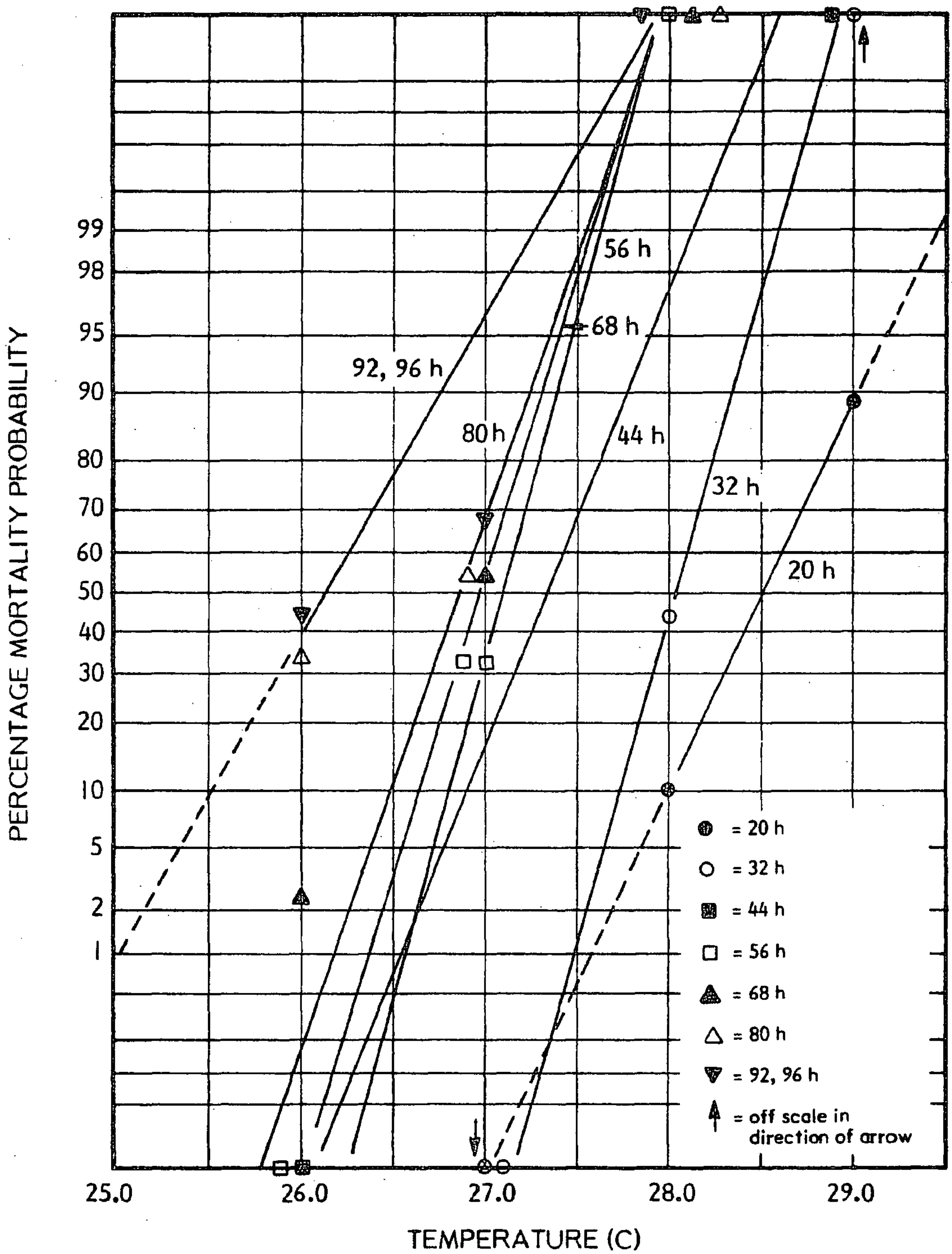

FIGURE 2.10.25-5

PERCENTAGE PROBABILITY MORTALITY VERSUS TEMPERATURE FOR 12 C ACCLIMATED ANIMALS: TEGULA BRUNNEA HEAT TOLERANCE

The lines were fitted by eye.

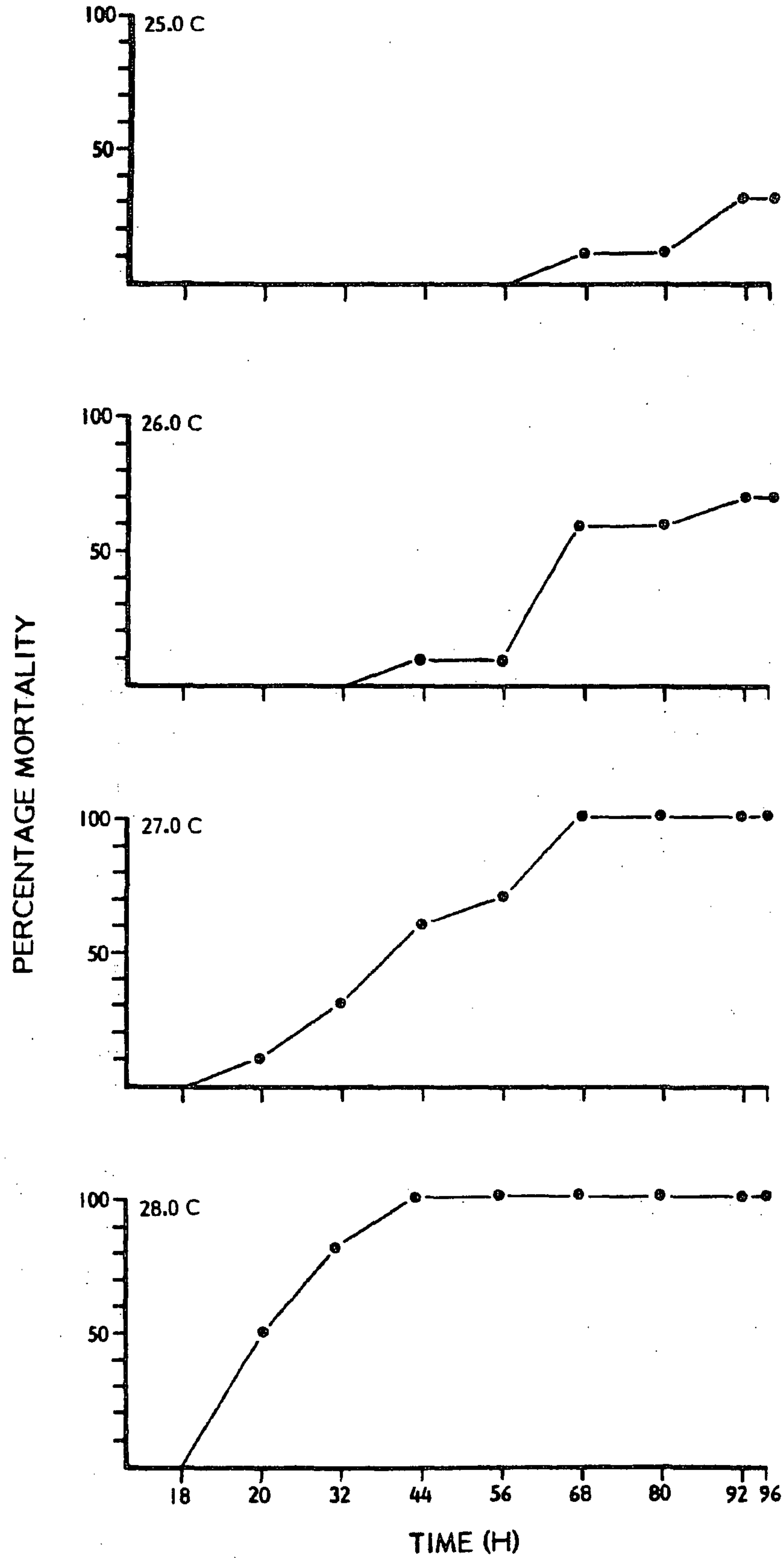

FIGURE 2.10.25-6

CUMULATIVE MORTALITY VERSUS TIME FOR 15 C ACCLIMATED ANIMALS: TEGULA BRUNNEA HEAT TOLERANCE

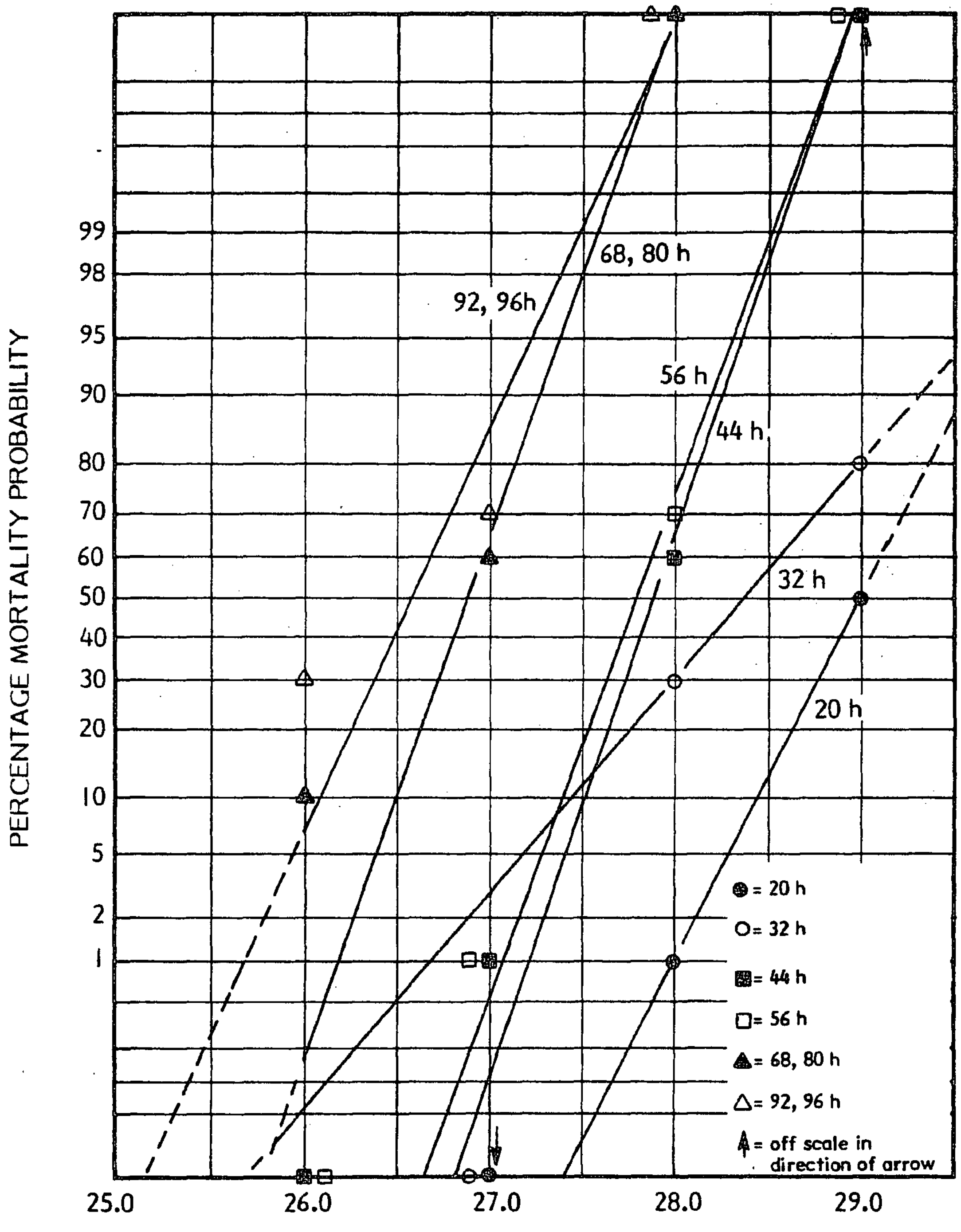

FIGURE 2.10.25-7

PERCENTAGE MORTALITY PROBABILITY VERSUS TEMPERATURE FOR 15 C ACCLIMATED ANIMALS: TEGULA BRUNNEA HEAT TOLERANCE

The lines were fitted by eye.

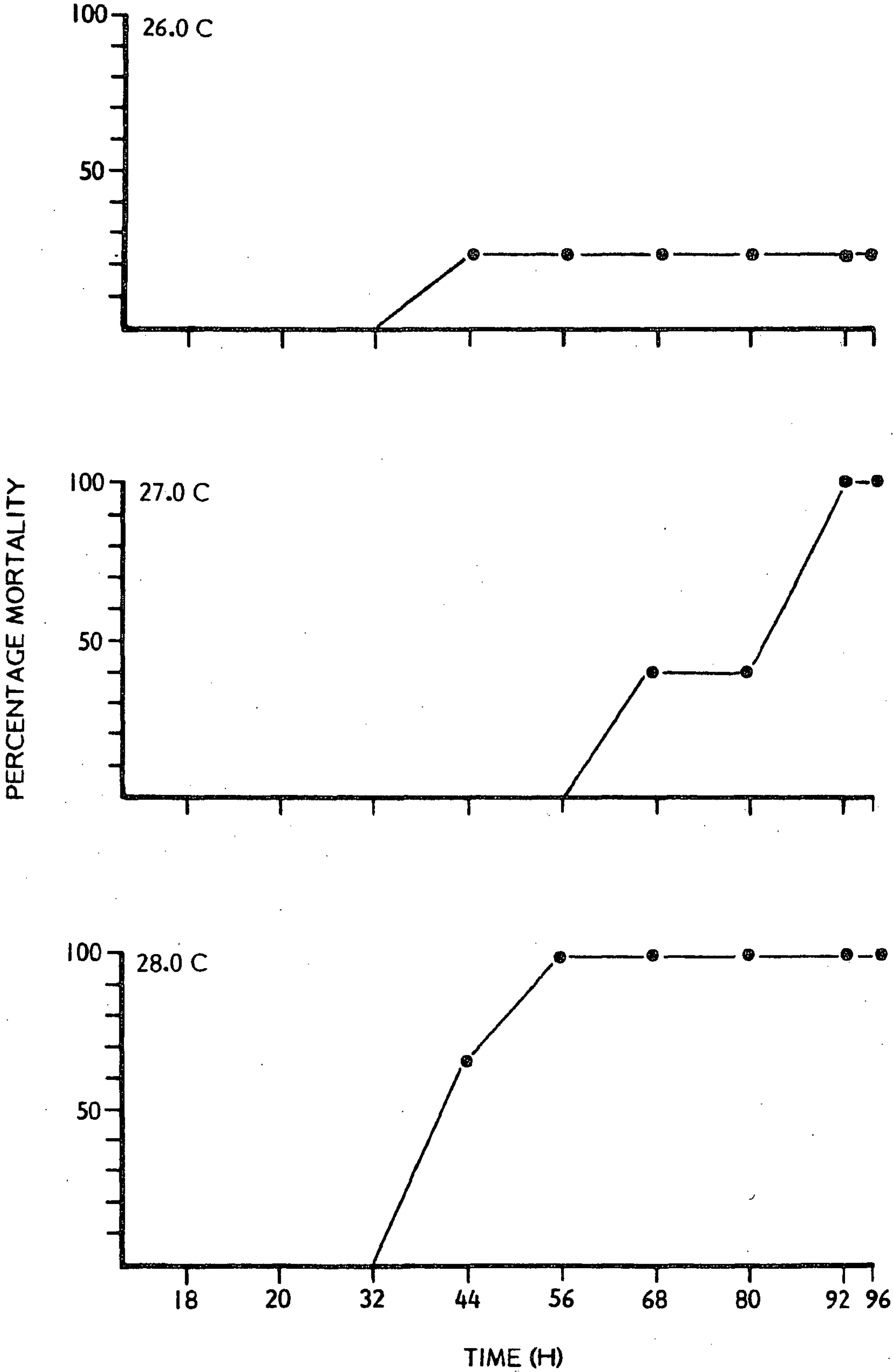

FIGURE 2.10.25-8

CUMULATIVE MORTALITY VERSUS TIME FOR 24 C ACCLIMATED ANIMALS: TEGULA BRUNNEA HEAT TOLERANCE

Probit regression lines were fitted by eye to the mortality data to determine the temperature which would cause 50 percent mortality at each observation period (FIGURE 2.10.25-9). The 96-hour ET50 value was determined to be 26.3C.

The estimators of the ET50 value thus calculated for each observation period were used to generate a curve of time to 50 percent mortality versus temperature, which is presented in FIGURE 2.10.25-10. The "incipient lethal" temperature, indicated by the vertical alignment of points to the left of the curve, was not defined by the experiment for any of the acclimation groups.

Discussion

Based on the results of this experiment, seawater temperatures in excess of 25.2C (77.4F) for four days would be expected to cause 50 percent mortality in a population of *Tegula brunnea* acclimated to 12C (54F). Seawater temperatures in excess of 25.6C (78.1F) for four days would be expected to cause 50 percent mortality in a population of *T. brunnea* acclimated to 15C (59F). Seawater temperatures in excess of 26.3C (79.3F) for four days would be expected to cause 50 percent mortality in a population of *T. brunnea* acclimated to 24C (75F). Little or no thermal mortality would be expected for exposures to temperatures at or below 24C (75F) for individuals acclimated to 12 to 24C (54 to 75F) for the same period of time. These results are in close agreement with results presented in the previous section on ambient (approximately 11-12C, 52-54F) acclimated *T. brunnea*.

The relationship of thermal response to acclimation temperature followed a logical trend. It is interesting to note that populations of *T. brunnea* can exist for extended periods of time with no thermal mortality within a few degrees of their upper lethal temperature.

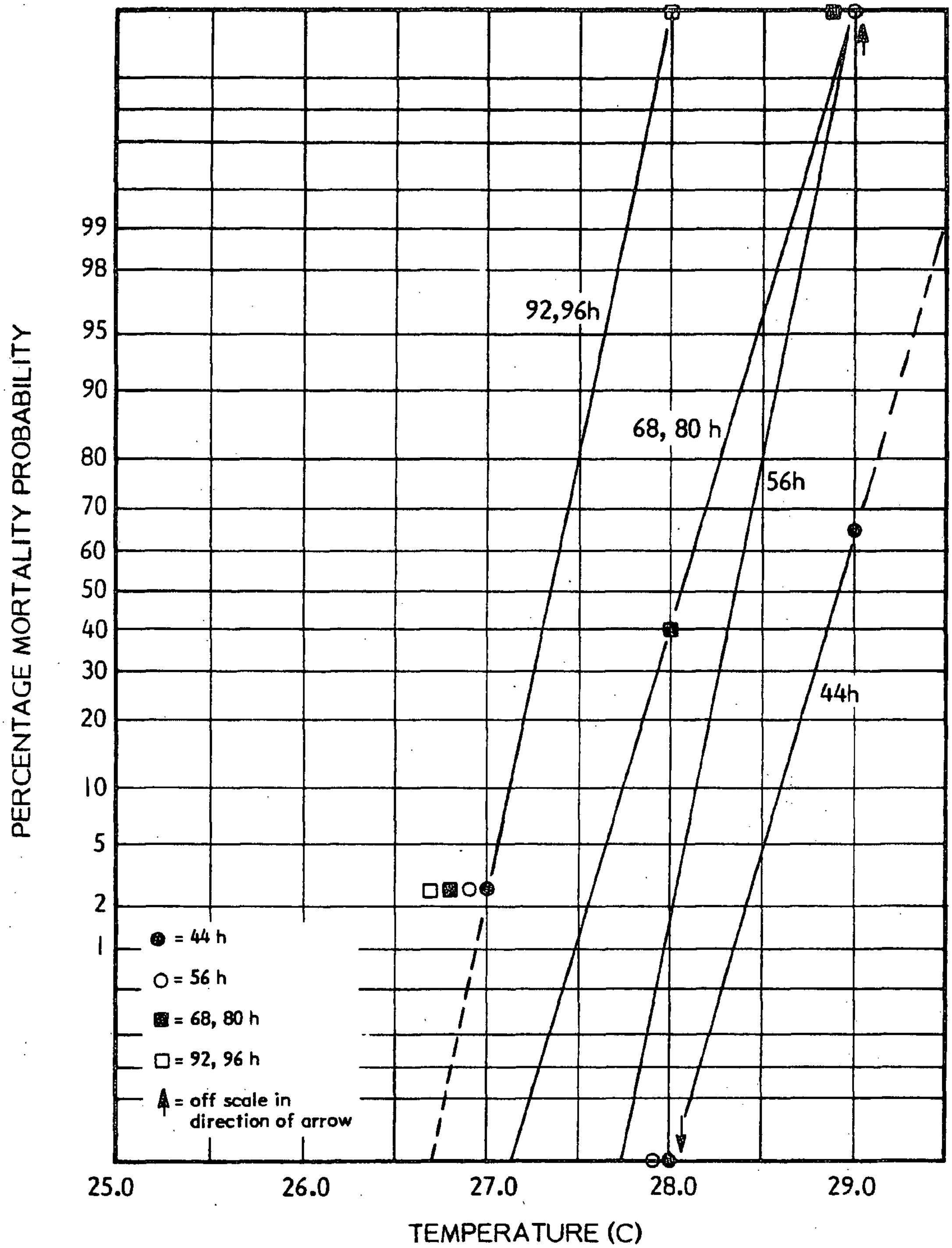

FIGURE 2.10.25-9

PERCENTAGE MORTALITY PROBABILITY VERSUS TEMPERATURE FOR 24 C ACCLIMATED ANIMALS: TEGULA BRUNNEA HEAT TOLERANCE

The lines were fitted by eye.

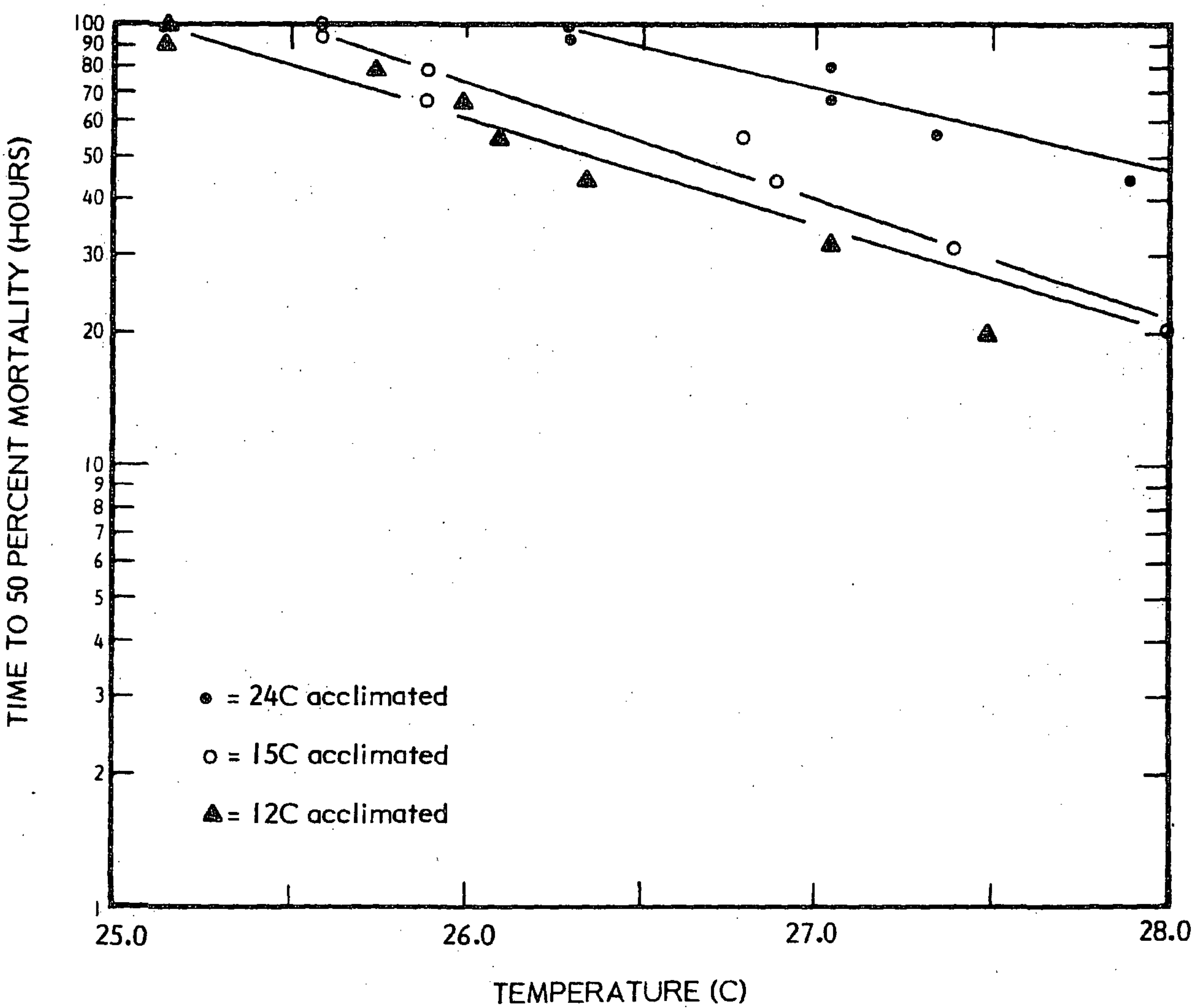

FIGURE 2.10.25-10

TIME TO 50 PERCENT MORTALITY VERSUS TEMPERATURE FOR THREE ACCLIMATION GROUPS: TEGULA BRUNNEA HEAT TOLERANCE

2.10.26 TEGULA FUNEBRALIS 96-HOUR MEDIAN EFFECTIVE TEMPERATURE (96 H ET50)

Tegula funebralis is not one of the RIS but it is a common snail in the Diablo Canyon area. It was incorporated into the 96-hour ET50 experiment along with the three RIS species. The results of a 96-hour test to determine the median effective temperature (ET50), i.e., the temperature at which 50 percent of the test population survived, are reported on in this section. The black turban snail ranges from Sitka, Alaska to Baja California, Mexico. It predominates in the upper intertidal (zone 2 of Ricketts et al. 1968) whereas the closely related RIS species, T. brunnea, predominates at lower levels. According to Burge and Schultz (1973) T. funebralis "was possibly the most abundant and ubiquitous algivore" in their intertidal surveys at Diablo Canyon, especially at high levels in protected areas. They concluded that a seasonal migration occurred in which the animals moved to higher levels during winter months, where they were observed to be feeding on drifted algae. To date, no published accounts of thermal tolerance studies on this species are available.

The study objective was to determine the 96-hour ET50 value of this species as an index to its thermal tolerance. In addition, the time course of mortality was recorded for the 96-hour period. The purpose of this study was to provide some indication of the possible effects of the power plant heated discharge on T. funebralis populations in Diablo Cove.

2.10.26.1 METHODS

A summary description of the laboratory methods employed in the investigations into heat tolerance of Tegula funebralis was presented in SECTION 2.4.2. For a more detailed description of the laboratory methods see APPENDIX B (Procedure 319) and protocol see APPENDIX C (experiment 19a).

The animals used in this experiment were greater than or equal to 15 mm in diameter and were collected in December 1978. They were then placed in the snail growth experiment (see SECTION 3.5.2) for 90 days prior to this experiment. Only animals from the ambient treatment of the growth experiment were used for the 96-hour ET50 experiment.

During the experiment, the animals were placed in cages made of 1 ft lengths of 3 in. diameter PVC pipe with 1/8 in. mesh plastic screening at both ends and with an airstone fitted inside the pipe. The cages were kept continuously submerged in the laboratory test tanks. Temperature data were recorded hourly by an Autodata Nine computer, transcribed, and summarized as cumulative means and standard deviations at each observation period for each test tank.

Test animals were observed at 12-hour intervals during the 96-hour period at test temperatures of 15.1 C and ranging from 23.1 to 28.8 C. A control group of animals was maintained and observed at ambient temperatures.

Failure of an animal to retract into its shell after prodding constituted the criterion for "ecological death" (effective temperature, ET) during the experiment. Animals which met this criterion were returned to ambient temperatures to observe any recovery. Nine or ten animals were tested in each treatment group.

2.10.26.2 RESULTS

Cumulative means and standard deviations of the temperature data for all test tanks are given in TABLE 2.4-6 (see SECTION 2.4.2).

Mortality was observed only in the 28.8 C tank, where 60 percent of the animals had died by the end of the 96-hour period.

All "dead" animals were replaced into ambient seawater to determine if they would recover. At 162 hours, none of the dead animals had recovered. All live animals at the end of the 96-hour test period were placed into ambient seawater to determine whether any "latent" mortality would occur. No latent mortalities were observed at 162 hours.

A graph of the cumulative mortalities in the 28.8 C tank is presented in FIGURE 2.10.26-1. Because so few intermediate mortality data points were obtained, further analysis does not appear to be warranted. Judging by the results, the 96-hour ET50 value for this species is probably slightly above 28.8 C.

2.10.26.3 DISCUSSION

Using the criterion of lack of response to prodding to define death, seawater temperatures exceeding 28.8 C (83.8 F) for four days would be expected to cause about 60 percent of the exposed population of _Tegula funebralis_ to die. A shorter exposure or lower temperatures would produce fewer mortalities and no mortality would be expected at and below 28.1 C (82.6 F) for a four day exposure.

Based on these results, the thermal tolerance of _T. funebralis_ is greater than that of _T. brunnea_, which is intuitively logical in that _T. funebralis_ is probably exposed to greater extremes of temperature in its natural habitat.

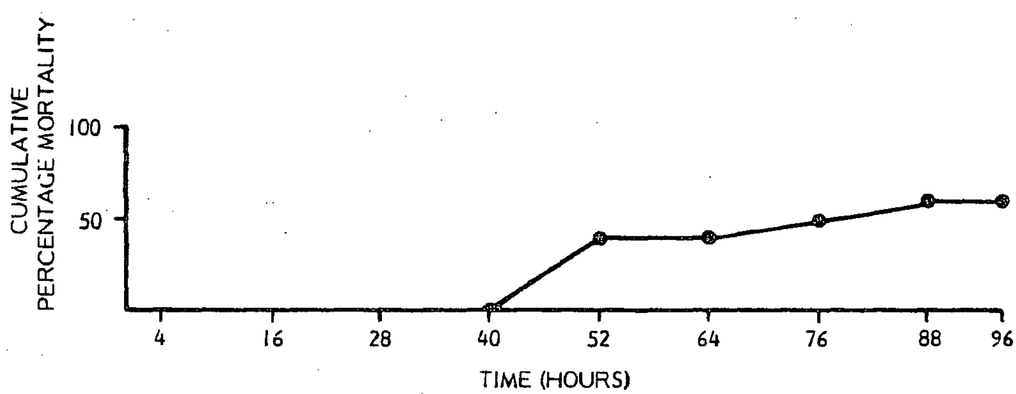

FIGURE 2.10.26-1

CUMULATIVE PERCENTAGE MORTALITY VERSUS TIME AT 28.8 C:
<u>TEGULA FUNEBRALIS</u> HEAT TOLERANCE

The animals were acclimated to ambient temperatures.

2.10.27 TONICELLA LINEATA 96-HOUR MEDIAN EFFECTIVE TEMPERATURE (96 H ET50)

The chiton Tonicella lineata is not one of the RIS but it occurs in the Diablo Canyon area. Because space was available, it was incorporated into the 96-hour ET50 experiment along with the three RIS species. The results of this 96-hour test to determine the median effective temperature (ET50), i.e. the temperature at which 50 percent of the test population survived, are reported on in this section.

As far as is known, there are no published accounts of thermal effects studies on Tonicella lineata. The study objective was to determine the 96-hour ET50 value of this species as an index to its thermal tolerance. In addition, the time course of mortality was recorded for the 96-hour period. The purpose of this study was to provide some indication of the possible effects of the power plant heated discharge on T. lineata populations in Diablo Cove.

2.10.27.1 METHODS

A summary description of the laboratory methods employed in the investigations into heat tolerance of T. lineata was presented in SECTION 2.4.2. For a more detailed description of the laboratory methods see APPENDIX B (Procedure 319) and protocol see APPENDIX C (experiment 19a). The animals used in this experiment were about 15-20 mm long and were collected subtidally just south of the power plant intake cove on 18 April 1979. The animals were assumed to be acclimated to ambient temperatures.

During the experiment the animals were maintained on "hanging habitats" which were suspended so that they were submerged but not touching any surrounding surface. The habitat consisted of a 6 in. diameter PVC pipe cut in half longitudinally and 6-8 in. wide.

Temperature data were recorded hourly by an Autodata Nine computer, transcribed, and summarized as cumulative means and standard deviations at each observation period for each test tank.

Test animals were observed at 12-hour intervals during the 96-hour period at test temperatures of 15.1 C and ranging from 23.1 to 28.8 C. A control group of animals was maintained and observed at ambient temperatures.

Loss of ability to hold a surface or failure to show a "curl" response constituted the criteria for "ecological death" (effective temperature, ET) during the experiment. Animals which met these criteria were returned to ambient temperatures to observe any recovery. Seven to fourteen animals were tested in each treatment group.

2.10.27.2 RESULTS

Cumulative means and standard deviations of the temperature data for all test tanks are given in TABLE 2.4-6 (see SECTION 2.4.2). No mortality was observed at 15.1 C. However, two mortalities occurred in the ambient tank at 64 hours. These are assumed not to be thermally induced deaths and are not considered further here. At 27.0, 28.1 and 28.8 C, 100 percent mortality was observed at the 4-hour observation period. Cumulative mortalities for all other test tanks are shown in FIGURE 2.10.27-1. All "dead" animals were replaced into ambient seawater to determine if they would recover. At 162 hours, none of the dead animals had recovered. All live animals at the end of the 96-hour test period were placed into ambient seawater to determine whether any "latent" mortality would occur. Only two latent mortalities were observed in the 23.1 C treatment group (resulting in 100 percent mortality) at 162 hours. These mortalities are not considered further in this report.

Probit regression lines were fitted by eye to determine the temperature at 50 percent mortality at each observation period. The 96-hour ET50 value could not be directly determined but was graphically estimated by extrapolation to be approximately 22.8 C, based on 83 percent mortality at 23.1 C and 100 percent

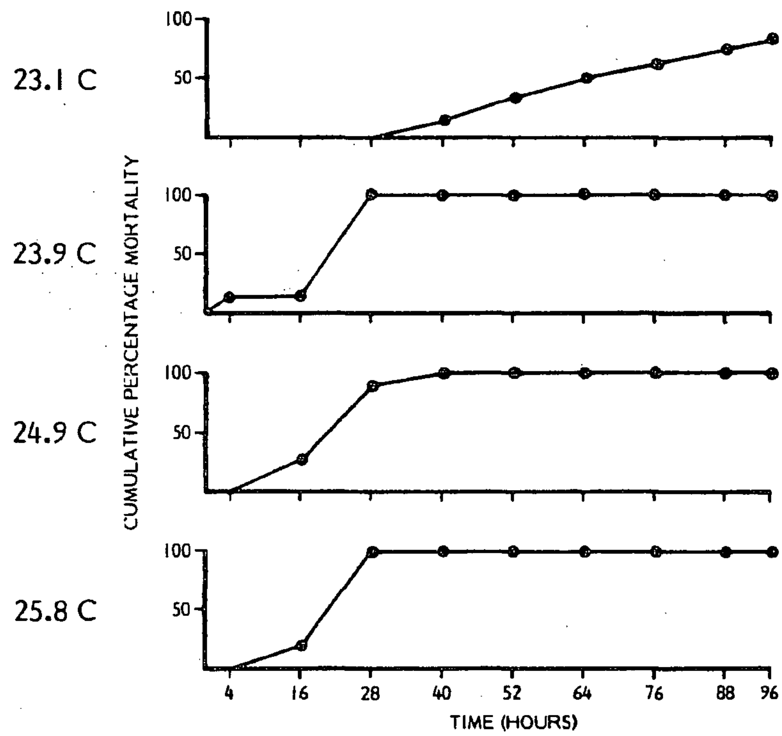

FIGURE 2.10.27-1

CUMULATIVE PERCENTAGE MORTALITY VERSUS TIME:
TONICELLA LINEATA HEAT TOLERANCE

The animals were acclimated to ambient temperatures.

mortality at 23.9 C. Estimations of the temperatures at which 50 percent mortality occurred at each observation period were derived from the probability plots and used to generate a curve of time to 50 percent mortality versus temperature shown in FIGURE 2.10.27-2. The "incipient lethal temperature" was not defined in this experiment.

2.10.27.3 DISCUSSION

Using the criteria of lack of ability to hold to a surface and absence of a "curl" response, seawater temperatures exceeding 22.8 C (73.0 F) for four days would be expected to cause approximately one half of the Tonicella lineata population to die. Temperatures at or above 27.0 C (80.6 F) would be expected to result in 100 percent mortality within a few hours. Shorter exposures and lower temperatures would produce fewer mortalities.

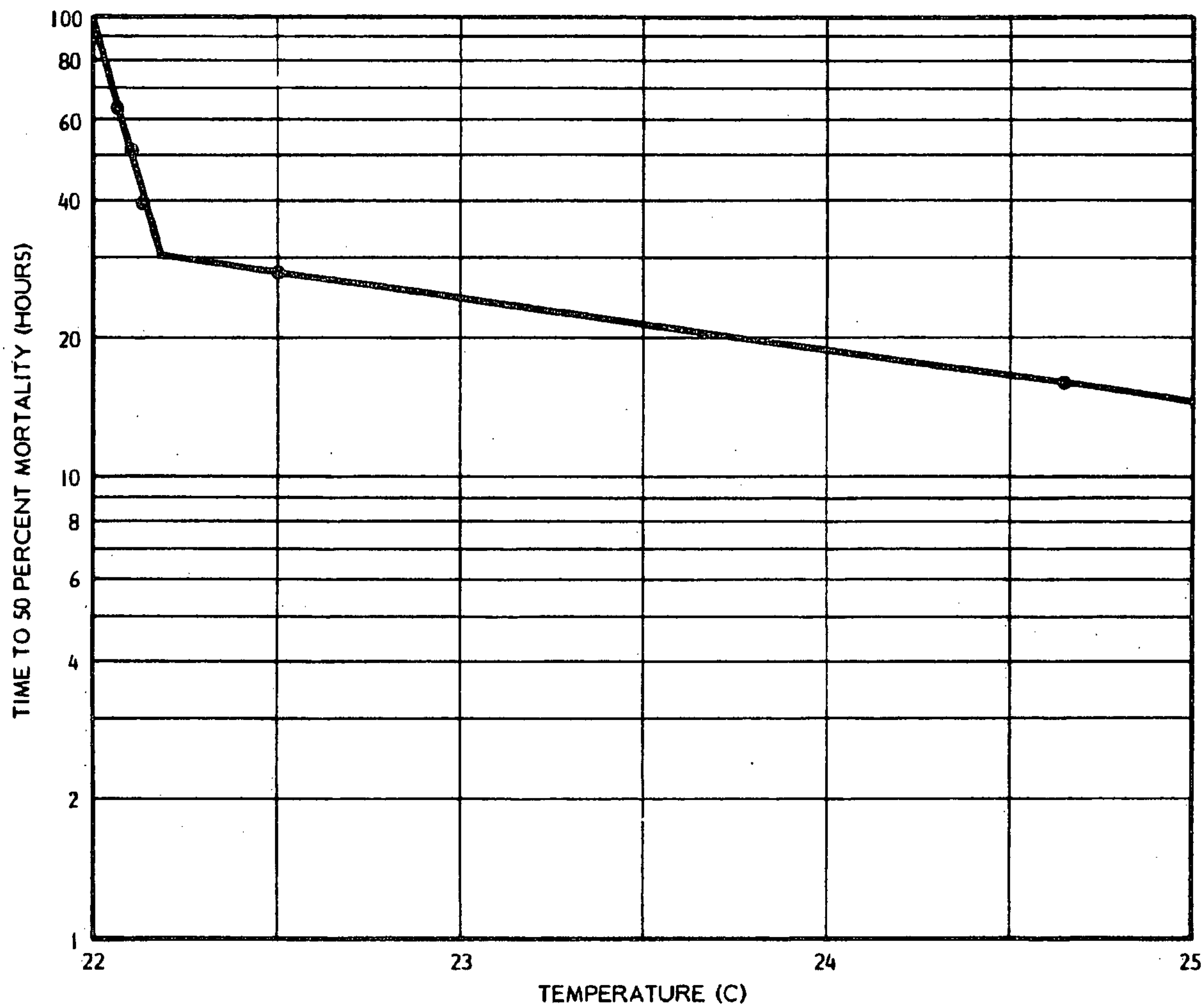

FIGURE 2.10.27-2

TIME TO 50 PERCENT MORTALITY VERSUS TEMPERATURE:
TONICELLA LINEATA HEAT TOLERANCE

The animals were acclimated to ambient temperatures.

2.10.28 BOTRYOGLOSSUM FARLOWIANUM 96-HOUR MORTALITY

Botryoglossum farlowianum is not an RIS but it occurs in the area of highest potential effect in Diablo Cove. It was the third most abundant algal category (the other two were the articulated coralline algae of the "CBS complex" and encrusting coralline algae) measured in percentage cover at the subtidal stations (10 and 15 ft depths) in Diablo Cove (PGandE 1979a).

This species is a red alga of the order Ceramiales which forms extensive beds in the shallow subtidal region. The plants are attached to the substrate by a "creeping" holdfast from which arise numerous upright axes up to 25 cm (10 in.) or more in length that are richly branched flattened blades. The life history is assumed to follow the typical triphasic pattern of many red algae in which the sexual plants alternate with asexual plants all of which are similar in morphology. The geographical distribution of the species is from Vancouver Island, British Columbia, Canada to Baja California, Mexico (Abbott and Hollenberg 1976). There are no publications on this species other than those dealing with morphological and taxonomic topics.

The study objective was to obtain some indication of the heat tolerance of this species by means of a relatively short treatment period. The purpose was to provide a basis for an estimation of the effects of the power plant thermal discharge on B. farlowianum populations in Diablo Cove.

2.10.28.1 METHODS

A summary description of the laboratory methods employed in the investigations into heat tolerance of B. farlowianum is presented below. For a more detailed description of the laboratory methods see APPENDIX B (Procedure 316) and protocol see APPENDIX C (experiment 16b).

Standard methodologies of studying thermal tolerance have not been developed for macroscopic marine algae. To study the thermal tolerance of B. farlowianum we adopted a 96-hour test period which is a generally accepted period for testing thermal tolerance of marine animals. In plants death is more difficult to define than it is in animals because small portions of a plant that survive treatment may in time regenerate an entire new plant. For this reason it was necessary to include in the methodology for algal species a post-treatment period during which the plants are returned to ambient temperatures for 96-hours to observe any reversal of temporary effects. The test on B. farlowianum was run simultaneously with that on Pterygophora californica (see SECTION 2.8). The temperatures used were ambient, 16, 19 21 and 24 C.

The plants used in this experiment were collected in the subtidal region at about 10 ft depth on 30 March 1979 from the area just south of the power plant intake cove. They were held in the laboratory at ambient temperatures and sorted to remove any portions of plants injured during collection. Intact upright thalli were divided into 100 plants ranging from 4-80 g wet weight which were subdivided into 5 groups of 20 individuals. Each individual was tagged with a numbered piece of plastic adhesive tape wrapped around the plant near the base and pressed together at the ends.

Following the start of the experiment when the plants were placed into the treatment tanks, 5 plants were removed from each tank at 24-hour intervals (i.e., 24, 48, 72 and 96-hour cohorts). Plants were examined prior to placement in the treatment tanks, when removed from the treatment tanks and at the end of the recovery period (216 or 240 hours). Each plant was photographed at each examination period. The following charactertistics were scored:

- o Bleaching of blade — total loss of photosynthetic pigments resulting in a whitish coloration, quantitatively scored by visual estimation as 0 = none, 1 = less than 25 percent of blade surface, 2 = less than 50 percent, 3 = less than 75 percent and 4 = total blade surface bleached
- o Disintegration of blade — using the same scoring system as for bleaching

- o Disintegration of stipe and holdfast — 0 = none, 1 = partial, 2 = complete
- o Change in pigmentation — 0 = normal, 1 = lighter than normal but not yet bleached
- o Turgor — 0 = normal, 1 = flaccid; based on rubbing of blade between the fingers

Illumination was provided by banks of 25 W Duro-Test Ultra-Hi Output Vita-Lights suspended above the raceway tanks providing 121-180 $\mu E/m^2/s$ for all treatments on a continuous (24-hour) photoperiod.

2.10.28.2 RESULTS

The statistical summary of the temperature data was presented in TABLES 2.8-1 and 2.8-2 (see SECTION 2.8 of this report). The mean values for the 96-hour period were approximately 11.7 (ambient tank), 16.2, 19.1, 21.0 and 23.8 C.

The observations on blade bleaching appeared to provide the best indication of response to temperature. Blade bleaching index scores (means of 5 plants) are plotted in FIGURE 2.10.28-1 by sample cohort for each treatment temperature. The change in index scores obtained at the end of the recovery period is indicated for each cohort sample by a vertical line. Except for a mean index score of 0.6 in the 24-hour cohort at the end of the recovery period, no bleaching was observed in the ambient treatment cohorts. Total blade bleaching resulted from exposure to 24 C by the end of the recovery period in all but the 24-hour cohort.

The data on blade disintegration at the end of the recovery period are presented in TABLE 2.10.28-1. A small amount of disintegration occurred in the ambient treatment 24-hour cohort and at 16.2 and 19.1 C treatments especially in the 96-hour cohort. Major disintegration had occurred in all cohorts of the 23.8 C treatment by the end of the recovery period.

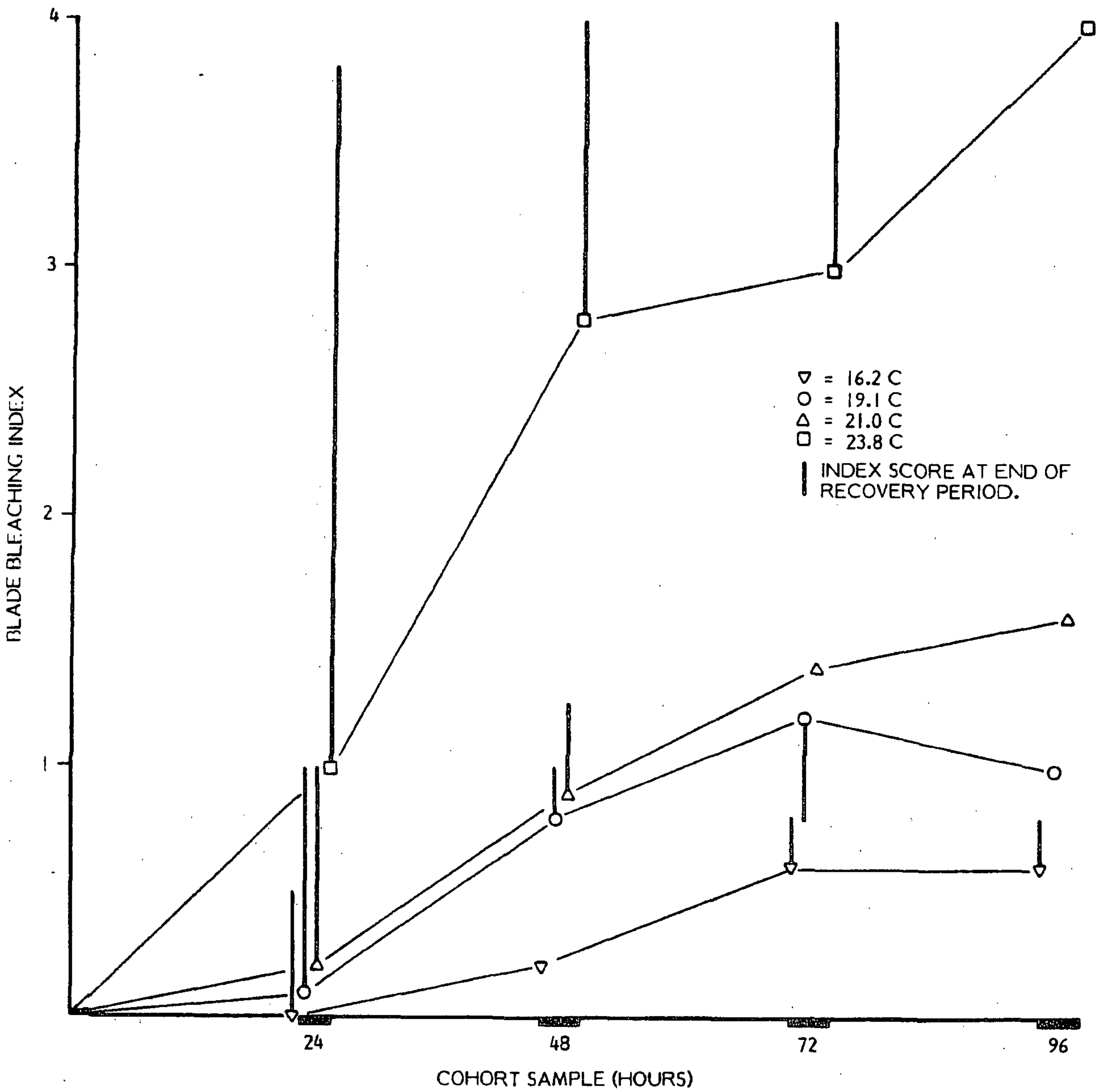

FIGURE 2.10.28-1

BLADE BLEACHING INDEX VERSUS TIME OF COHORT SAMPLE: BOTRYOGLOSSUM FARLOWIANUM HEAT TOLERANCE

A higher bleaching index indicates more bleaching.

2.10.29 CALLIARTHRON TUBERCULOSUM 216-HOUR MORTALITY

Calliarthron tuberculosum is not an RIS but it occurs in the area of highest potential effect in Diablo Cove. Because sufficient space was available in the tanks, it was decided to include C. tuberculosum in the heat tolerance experiment conducted on Pterygophora (see SECTION 2.8). Quantitatitive data on this species collected by the 316(a) program are included in the sampling category "CBS complex" which also includes species of Bossiella and Serraticardia. Calliarthron probably makes up at least one half of the percentage cover values in the CBS complex data from subtidal samples. The CBS complex was the most abundant category of algae (as percentage cover) at the 10 and 15 ft depths in Diablo Cove (PGandE 1979a).

This species is a red alga of the family Corallinaceae (coralline algae) which is mostly calcareous but with uncalcified joints that allow some bending of the upright portions. They are attached initially by a basal crust from which are produced numerous upright fronds 20 cm (8 in.) or more tall that are richly branched. An unusual means of vegetative propagation in this species results from the ability of the tips of the upright fronds to become attached to a substrate, forming a new "basal crust" from which more upright fronds can arise (Johansen and Austin 1970, P. A. Lebednik, 316(a), unpublished observations). The species ranges from Alaska to Isla Cedros, Baja California, Mexico and is often extremely common in low tidepools or subtidally in kelp beds (Abbott and Hollenberg 1976). The life history is assumed to follow the typical triphasic pattern of many red algae in which the sexual plants alternate with asexual plants all of which are similar in morphology. There are no publications on this species dealing with thermal tolerance.

The study objective was to obtain some indication of the heat tolerance of this species by means of a relatively short treatment period. The purpose was to provide a basis for an estimation of the effects of the power plant thermal discharge on C. tuberculosum populations in Diablo Cove.

2.10.29.1 METHODS

A summary description of the laboratory methods employed in the investigations into heat tolerance of C. tuberculosum was presented in SECTION 2.8. For a more detailed description of the laboratory methods see APPENDIX B (Procedure 316) and protocol see APPENDIX C (experiment 16d).

Standard methodologies of studying thermal tolerance have not been developed for macroscopic marine algae. To study the thermal tolerance of C. tuberculosum, we originally planned a 96-hour treatment period. However, there were few effects observed at the end of 96 hours and so the treatment period was extended to 216 hours.

The plants used in the experiment were collected on 30 March 1979 in the shallow subtidal region from the area just south of the power plant intake cove. They were held in the laboratory at ambient temperatures and sorted to remove any portions of plants injured during collection. Intact upright thalli were sorted into 25 plants, each 5-15 cm long, which were subdivided into five groups of five individuals. Each individual was tagged with a numbered piece of plastic adhesive tape wrapped around the plant near the base and pressed together at the ends. The plants were examined for a set of characteristics just prior to placement in the treatment tanks and again at the end of the 216-hour period. The following characteristics were examined:

- o Bleaching of frond — total loss of photosynthetic pigments resulting in a whitish coloration, quantitatively scored by visual estimation as 0 = none, 1 = less than 25 percent of frond surface, 2 = less than 50 percent, 3 = less than 75 percent and 4 = total blade surface bleached
- o Disintegration of frond — using the same scoring system as for bleaching
- o Change in pigmentation — 0 = normal, 1 = lighter than normal but not yet bleached

Illumination was provided by banks of 25 W Duro-Test Ultra-Hi Output Vita-Lights suspended above the raceway tanks providing 121-180 μ E/m^2/s for all treatments on a continuous (24-hour) photoperiod.

A recovery period was not included in the experiment on C. tuberculosum.

2.10.29.2 RESULTS

The statistical summary of the temperature data for the first 96 hours of the treatment period was presented in TABLES 2.8-1 and 2.8-2 (see SECTION 2.8 of this report). Temperatures for the rest of the 216-hour period were quite comparable and are not reported here. The mean values for the 96-hour period were approximately 11.7 (ambient), 16.2, 19.1, 21.0 and 23.8 C. These mean values will be used to identify the treatments in this section for clarity and ease of comparison.

At the end of the 216-hour treatment period, all plants in the ambient, 16.2 and 19.1 C treatments appeared normal. In the 21.0 C treatment, a slight lightening of color was observed in all of the plants but this was not sufficient to merit a score of 1 in the pigmentation category. Otherwise, the plants appeared normal. Some bleaching (less than 25 percent of frond area) and lightening of pigmentation (scored as 1) was observed in all plants of the 23.8 C treatment group.

2.10.29.3 DISCUSSION

Calliarthron tuberculosum does not exhibit any response to temperatures up to 19.1 C (66.4 F) and at 21.0 C (69.8 F), only a slight lightening of pigmentation is evident after 216 hours of exposure. A temperature of 23.8 C (74.8 F) induces some bleaching and general lightening of pigmentation after 216 hours. Because Calliarthron, in common with many coralline algae, exhibits a slow rate of growth (Johansen and Austin 1970), it may respond rather slowly to environmental changes such as the elevated temperatures used in this experiment. It is possible that a longer exposure than 216 hours would result in more bleaching at 23.8 C (74.8 F) and possibly also at 21.0 C (69.8 F).

2.10.30 GASTROCLONIUM COULTERI 216-HOUR MORTALITY

A review of the literature on the RIS red seaweed Gastroclonium coulteri was presented in PGandE (1979a). Because sufficient space was available in the tanks, it was decided to include G. coulteri in the heat tolerance experiment conducted on Pterygophora (see SECTION 2.8) and to extend the treatment time to 216 hours.

Gastroclonium coulteri is the fourth or fifth most abundant algal species in the Diablo Canyon area at the +1 ft tide level (PGandE 1978). This species typically forms a low turf of intertwined basal stolons and upright branched thalli, making it an important "habitat former" for invertebrates at low intertidal levels. There are no publications on thermal tolerance of this species. The geographical distribution (southern British Columbia, Canada to Baja California, Mexico according to Abbott and Hollenberg (1976) indicates that this species may tolerate relatively high seawater temperatures. Nevertheless, it was decided to study its heat tolerance because of its importance and abundance in the area of highest potential effect in Diablo Cove.

The study objective was to obtain some indication of the heat tolerance of the species by means of a relatively short treatment period. The purpose was to provide a basis for an estimation of the effects of the power plant thermal discharge on G. coulteri populations in Diablo Cove.

2.10.30.1 METHODS

A summary description of the laboratory methods employed in the investigations into heat tolerance of G. coulteri was presented in SECTION 2.8. For a more detailed description of the laboratory methods see APPENDIX B (Procedure 316) and protocol see APPENDIX C (Experiment 16c).

Standard methodologies of studying thermal tolerance have not been developed for macroscopic marine algae. To study the thermal tolerance of G. coulteri, we originally planned a 96-hour treatment period. However, there were few effects observed at the end of 96-hour and so the treatment period was extended to 216 hours.

The plants used in the experiment were collected on 30 March 1979 in the shallow subtidal region from the area just south of the power plant intake cove. They were held in the laboratory at ambient temperatures and sorted to remove any portions of plants injured during collection. Intact upright thalli were sorted into 25 plants, each 5-15 cm long, which were subdivided into five groups of five individuals. Each individual was tagged with a numbered piece of plastic adhesive tape wrapped around the plant near the base and pressed together at the ends.

The plants were examined for a set of characteristics just prior to placement in the treatment tanks and again at the end of the 216-hour period. The following characteristics were examined:

- o Bleaching of blade — total loss of photosynthetic pigments resulting in a whitish coloration, quantitatively scored by visual estimation as 0 = none, 1 = less than 25 percent of blade surface, 2 = less than 50 percent, 3 = less than 75 percent and 4 = total blade surface bleached
- o Disintegration of blade — using the same scoring system as for bleaching
- o Disintegration of stipe and holdfast — 0 = none, 1 = partial, 2 = complete
- o Change in pigmentation — 0 = normal, 1 = lighter than normal but not yet bleached

Illumination was provided by banks of 25 W Duro-Test Ultra-Hi Output Vita-Lights suspended above the raceway tanks providing 121-180 $\mu E/m^2/s$ for all treatments on a continuous (24-hour) photoperiod.

A recovery period was not included in the experiment on G. coulteri.

2.10.30.2 RESULTS

The statistical summary of the temperature data for the first 96 hours of the treatment period was presented in TABLES 2.8-1 and 2.8-2 (see SECTION 2.8 of this report). Temperatures for the rest of the 216-hour period were quite comparable and are not reported here. The mean values for the 96-hour period were approximately 11.7 (ambient), 16.2, 19.1, 21.0 and 23.8 C. These mean values will be used to identify the treatments in this section for clarity and ease of comparison.

No bleaching of upright blades was observed during the experiment. Disintegration of the blade did occur in the 23.8 C treatment in that the lower lateral branchlets disintegrated on all plants; however, the upper lateral branchlets appeared to be healthy and growing. All of the 21.0 C treatment group exhibited the same response. The same phenomenon, involving only a few lower laterals, was observed in the ambient treatment.

Obvious lightening of pigmentation was observed in the 23.8 C treatment group, especially at the tips of upper laterals, but otherwise the plants appeared to be healthy. No other responses were observed by the end of the experiment.

2.10.30.3 DISCUSSION

Temperatures of 19.1 C (66.4 F) and lower for 216 hours do not appear to have an effect on G. coulteri. No complete mortality was observed at the highest treatment temperature 23.8 C (74.8 F).

Although some disintegration of lower laterals occurred in the 21.0 (69.8 F) and 23.8 C (74.8 F) treatments, it is not clear whether this effect would result in complete detachment or death of the plants inasmuchas the remaining portions appeared to be normal. The disintegration observed may have represented normal limited senescence which was hastened by elevated temperatures. It seems likely that temperatures of 23.8 C (74.8 F) and lower for 216 hours will not result in thermal mortality in G. coulteri.

2.10.31 SURFGRASS (PHYLLOSPADIX SCOULERI)

The surfgrass (Phyllospadix scouleri) forms dense beds along exposed areas of the California coast at the low water mark. These plants are perennials whose mats extend over large areas of the low intertidal region in Diablo Cove. As an abundant species near the low water line, it has a major influence on the ecology of the low intertidal communities by providing habitat in its foliage. Invertebrate communities inhabit its mat-like root system and a number of seaweed and invertebrate species also settle and grow upon the long slender leaves. Surfgrass may provide a "nursery" for the junvenile stages of various crustaceans (including the rock crab) and certain fishes.

The surfgrass is one of a number of flowering plant species (unrelated to the true grasses) that has become adapted to the marine environment. Numerous narrow straplike leaves, 1-3 ft long, arise from a densely branched root system that is firmly attached to the rock substrate. As denoted by its name, this species appears to be adapted to areas exposed to moderate to high wave action and it is limited to the lower intertidal and shallow subtidal zones, centered around the low water mark. The geographical distribution of this species ranges from Alaska to Santa Monica, California (see map). The surfgrass has a life cycle essentially identical to that of its terrestrial relatives; however, the seeds show interesting adaptations to the habitat of the mature plants. The seeds are buoyant and upon release are dispersed by means of surface currents onto the shore. Unique barbs are borne on the seed coats which very efficiently attach them upon contact to certain seaweed species (Turner 1981). Once attached, the seed germinates and a root grows down to the rock substrate after which the plant is no longer dependent upon the seaweed for attachment. Very little is known of the interactions between surfgrass and other marine species. Two studies (Stewart et al. 1978, Stewart and Myers 1980) have shown that surfgrass populations provide a habitat for many invertebrate and seaweed species in the open spaces of the root system and on the surface of the leaves. A number of nearshore fishes are also found in surfgrass beds, particularly juveniles of these species. Nothing is known of the growth rates of surfgrass nor of the consumption of this plant by invertebrate or fish species.

The results of an experiment to assess the heat tolerance of surfgrass are reported in this section. The objective was to determine whether surfgrass experiences any mortality after exposure to 24 C. The purpose was to provide information on the effects of the thermal discharge from the Diablo Canyon Power Plant on the surfgrass populations of Diablo Cove. A preliminary run of this experiment was conducted on 29 March 1979 when no formal records were kept. A second run, reported in detail here, was made to provide documentation of the results. The results of the two runs were identical.

2.10.31.1 METHODS

The procedure used in this experiment was modified from Procedure 316 (see APPENDIX B). The protocol is included in APPENDIX C (experiment 16e). Specimens of surfgrass were collected on 2 April 1982 from an intertidal area immediately south of Diablo Cove. The ambient seawater temperature at the time of collection was 11.4 C. The specimens were immediately taken to the laboratory and the experiment was begun without delay. For the purposes of this experiment, a clump of surfgrass consisting of approximately 5-10 cm diameter rhizome system with attached stems and leaves represented one "plant." Ten plants each were placed in an experimental raceway at 24 C and a control raceway at ambient seawater temperature (see APPENDIX A for facility description).

The test and control plants were observed at the beginning and end of the experiment (total of 9 days). Because the experiment ran for 9 days, by which time responses should have been evident, a recovery period was not included in this run. The primary response anticipated was bleaching of the leaves. At each observation, the plants were checked carefully for leaf bleaching but they were also examined for other evidences of temperature effects.

2.10.31.2 RESULTS

Over the 9-day test period, the mean temperature of the test tank was 24.3 C and that of the control tank was 11.7 C (see TABLE 2.10.31-1). Because of a thermistor malfunction in the test tank, the seawater temperature rose to about 35 C and remained at that temperature for three hours. Light intensity was continuous during the experiment, at approximately 120-160 μ E/m^2/s.

The ends of the leaves of one of the plants in the test tank were bleached and somewhat eroded at the beginning of the experiment and a slight increase in this bleaching and erosion was observed during the experiment. This almost certainly resulted from the plant's condition prior to collection and is not attributable to the test temperature. None of the other plants in either the test or control tanks exhibited bleaching or other apparent changes from their appearance at time of collection. Noticeable fungal growth was observed on the cut surfaces of the rhizomes of three of the plants in the test tank.

It was noted that the epiphytes on the surfgrass, in particular the blades of Smithora naiadum, were completely bleached after several days in the test tank while the plants in the control tank appeared normal. This probably would not have a detrimental effect on the surfgrass.

2.10.31.2 DISCUSSION

The populations of surfgrass at Diablo Cove can tolerate constant seawater temperatures of at least 24 C (75 F) for 9 days without exhibiting deleterious responses. In addition, because of an equipment malfunction, it was determined that they can tolerate a 3-hour exposure to 34 C (93 F).

TABLE 2.10.31-1

TEMPERATURE SUMMARY FOR TEST AND CONTROL TANKS: SURFGRASS HEAT TOLERANCE

Tank	Temperatures (C)		
	Mean	Standard Deviation	Range
Test[a]	24.3	1.1	21.9-25.4
Control	11.7	0.6	9.6-12.8

a Due to a 22-hour thermistor malfunction starting at 141 hours into the experiment, the temperature in the test tank dropped to about 11.5 C for 10 hours and then increased to about 34.7 C for 3 hours. These data are not included in the above statistics.