#3016

COMPENDIUM OF THERMAL EFFECTS LABORATORY STUDIES

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TERA CORPORATION

2150 Shattuck Avenue
Berkeley, California 94704
415·845·5200

Berkeley, California
Dallas, Texas
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3.0 GROWTH AND EARLY DEVELOPMENT

The results of experiments to determine the optimum temperatures for growth and early development are included in this section.

The long-term effects of increased temperature (sublethal or chronic effects) on species populations can be studied by means of experiments designed to determine the optimum temperature for growth. Below temperatures that are lethal to individuals of the species, the rate of growth may be influenced by changes in temperature. As an index to the physiological makeup of the species, the optimum temperature for growth may indicate that temperature to which the species is genetically adapted. Hence, assuming that optimal growth rates provide an indication of general well being of an individual, its survivability in nature may be enhanced at the optimal temperature for growth. Care must be taken in applying these generalizations to natural populations because certain features of the natural environment other than temperature can be suboptimal, resulting in lower growth rates. The primary factors influencing growth in animals are food composition and availability and in plants, light quality, intensity and duration. By designing experiments incorporating more than one diet (light regime), the optimum temperature for growth can be defined more completely.

Certain stages in the early development of organisms may have a very narrow range of temperature requirements, outside of which lack of development or lethal abnormalities may occur. It is generally assumed that the most sensitive stages are the very earliest in the development of the organism, i.e., embryological and larval stages in animals and spore or zygote germination and initial development stages in plants.

Given a condition in which temperatures are elevated but below the lethal limit for juveniles or adults, detrimental effects on early development may result. Such effects could range from complete mortality to a relatively low percentage of mortality at early developmental stages. This theoretical effect would not exist until reproduction was initiated in the exposed population. The timing of

reproduction in nature is usually quite seasonal for most species. The influence of detrimental effects in early developmental stages in adult populations would depend upon the recruitment source and plankton dispersal factors determining exposure to a fixed point thermal plume and in any event, would not become manifested in adult populations until that age class would theoretically have replaced the extant adult populations. Such population effects would not become evident until months or years after the early stages were affected. For these reasons, laboratory experimental studies on early development are the most efficient way to estimate possible thermal effects.

The results of growth experiments on four species each of fish, invertebrates and algae are presented in this section. Also presented are results from early development experiments on one species of fish, two species of invertebrates and five species of algae.

3.1 BLACK-AND-YELLOW/GOPHER ROCKFISH (SEBASTES CHRYSOMELA CARNATUS) GROWTH

The results of an experiment to determine the optimum temperature for growth of black-and-yellow/gopher rockfish (Sebastes chrysomelas/carnatus) is reported in this section.

Juvenile specimens of these two species were tested together because they cannot be differentiated until they begin to assume the coloration characteristics of the adult phase. Gopher rockfish were designated representative important species (RIS) for the Diablo Canyon 316(a) demonstration. The literature on this species was reviewed in a previous report (PGandE 1979b). The study objective of the optimum temperature for growth experiment on black-and-yellow/gopher rockfish was to determine the temperature or range of temperatures which produce maximum growth under laboratory conditions. The purpose of the experiment was to obtain an estimate of the effects of the power plant thermal plume on long-term growth of black-and-yellow/gopher rockfish juveniles.

3.1.1 METHODS

A summary of the methods used in this experiment is presented below. For a more detailed description of the methods used see APPENDIX B (Procedure 320) and for the experiment protocol see APPENDIX C (experiment 20b).

The individuals used in this experiment were collected in July and August 1978. All fish were taken from the northern end of Morro Bay, adjacent to the intakes of the PGandE Morro Bay Power Plant, using a 3 m otter trawl. The fish were transported to the Thermal Effects Laboratory and held there in 4 x 8 x 2 ft fiberglass tanks maintained at ambient seawater temperature. Just prior to the commencement of the experiment 30 fish were transferred to each of five black plastic tubs of approximately 100 liter (26.5 gal) capacity, one tub for each temperature condition. The transfer of experiment animals was followed by a "temperature adjustment" period during which temperatures were raised or

lowered at a rate of 1 C per day until the experiment temperature was reached in each tub. Target temperatures were 12, 16, 18, and 20 C with an additional tub maintained at the fluctuating ambient seawater temperature. Once an experiment test temperature was achieved, the fish were allowed to "acclimate" to that temperature for five days. Following this acclimation period, all 30 fish in a test tank were anesthetized with MS-222 and their individual standard length and wet weight were measured and recorded. These initial measurements marked the start of the experiment (elapsed days: 0) for that particular tank. The test populations were measured between 30 April and 8 May 1979. Measurements were repeated approximately every 30 days for the next 7 months (212 to 219 elapsed days).

The fish were fed an excess ration of frozen brine shrimp (Artemia) daily between 0900 and 1100 for the duration of the experiment. On days when measurements were made, feeding was postponed until after the data were collected. Growth rates were calculated using the formula for exponential growth (Brett et al. 1969): $w = be^{kt}$ (where w=weight, t=time and b and k are constants). When ln w is plotted against t, the slope k x 100 is equal to the growth rate. Tank temperatures were recorded hourly by the laboratory's Autodata Nine data acquisition system. Initially it was necessary to hand transcribe these data for subsequent analysis, but starting in October 1979 the Autodata Nine computer was interfaced with an Alpha Micro computer system allowing the temperature data to be recorded and summarized automatically.

3.1.2 RESULTS

Summary temperature and growth data for the experiment are presented in TABLE 3.1-1. The mean weights of the individual test populations, which were calculated following each observation (approximately every 30 days), are graphically presented in FIGURE 3.1-1. The individual weights recorded for each test population were log transformed, pooled from all observations, and plotted versus time. A least squares linear regression line was calculated for each population from these data. The regression lines are presented in FIGURE 3.1-2. The slope of each regression line, multiplied by 100, is equal to the specific

TABLE 3.1-1

SUMMARY OF TEMPERATURE AND GROWTH DATA: BLACK-AND-YELLOW/GOPHER ROCKFISH OPTIMUM TEMPERATURE FOR GROWTH

Target Temperature (C)	Ambient	12	16	18	20
Mean Temperature (C)*	13.2 ± 2.8	12.1 ± 0.3	16.1 ± 0.3	18.0 ± 0.4	20.0 ± 0.7
Start Date	30 April 79	1 May 79	4 May 79	7 May 79	8 May 79
Elapsed Time (Days)	0	0	0	0	0
Mean Standard Length (mm)	70 ± 3	69 ± 3	67 ± 3	69 ± 2	70 ± 3
Mean Wet Weight (g)	10.2 ± 1.4	9.8 ± 1.2	8.9 ± 1.3	10.0 ± 1.0	10.1 ± 1.3
N	30	30	30	30	30
Finish Date	28 November 79	29 November 79	4 December 79	11 December 79	13 December 79
Elapsed Time (Days)	212	212	214	218	219
Mean Standard Length (mm)	90 ± 5	87 ± 5	82 ± 3	83 ± 2	81 ± 3
Mean Wet Weight	25.1 ± 4.4	20.6 ± 4.0	18.4 ± 2.0	19.5 ± 2.2	17.3 ± 2.2
N	15	14	26	29	21
Growth Rate (% Δ W/Day)	.45 ± .07	.37 ± .06	.34 ± .06	.29 ± .05	.25 ± .06

Temperature data are ± 2 S.D; all other data are ± 2 S.E.

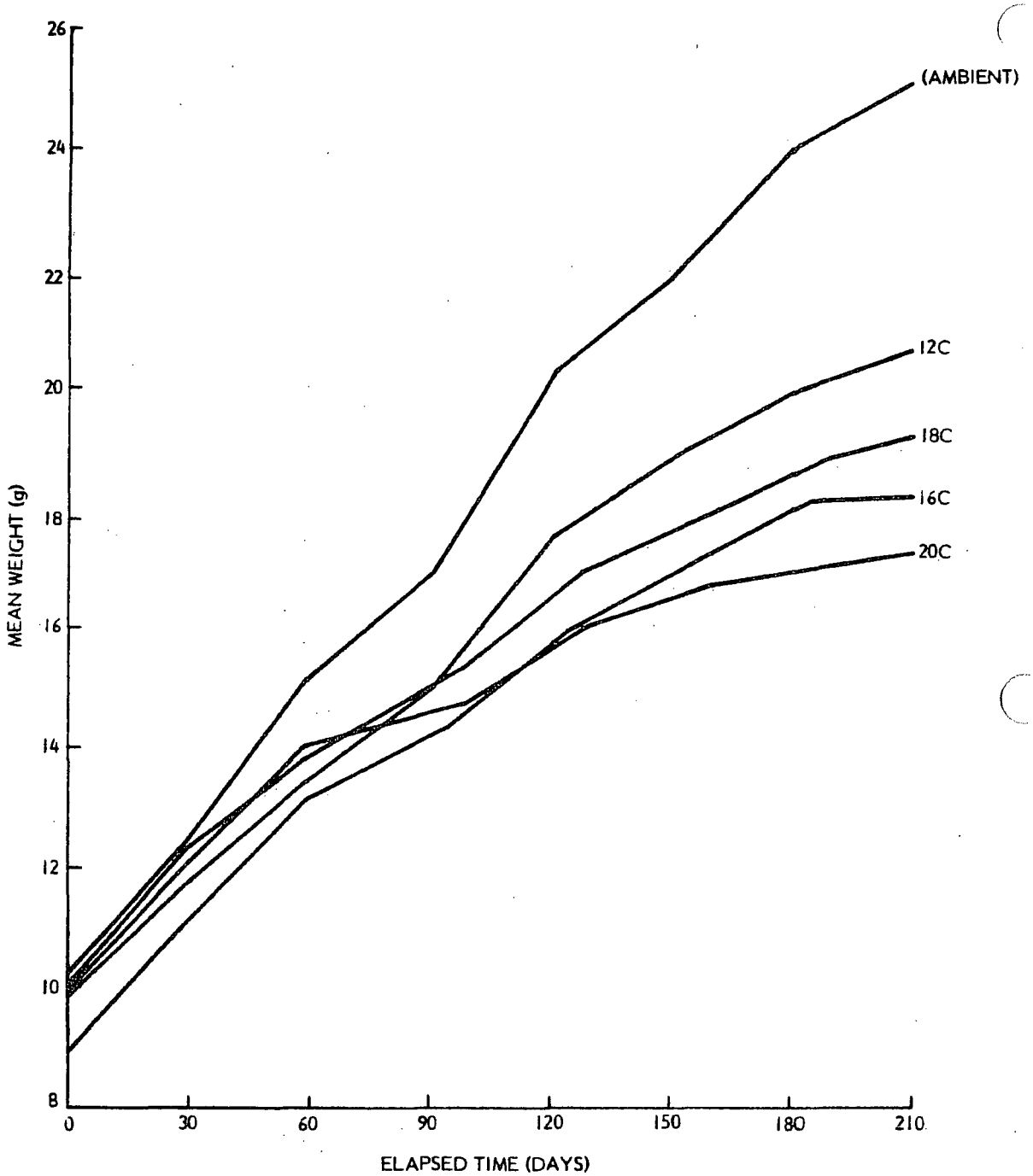


FIGURE 3.1-1

MEAN WEIGHT VERSUS TIME AT FIVE TEMPERATURES:
BLACK-AND-YELLOW/GOPHER ROCKFISH GROWTH

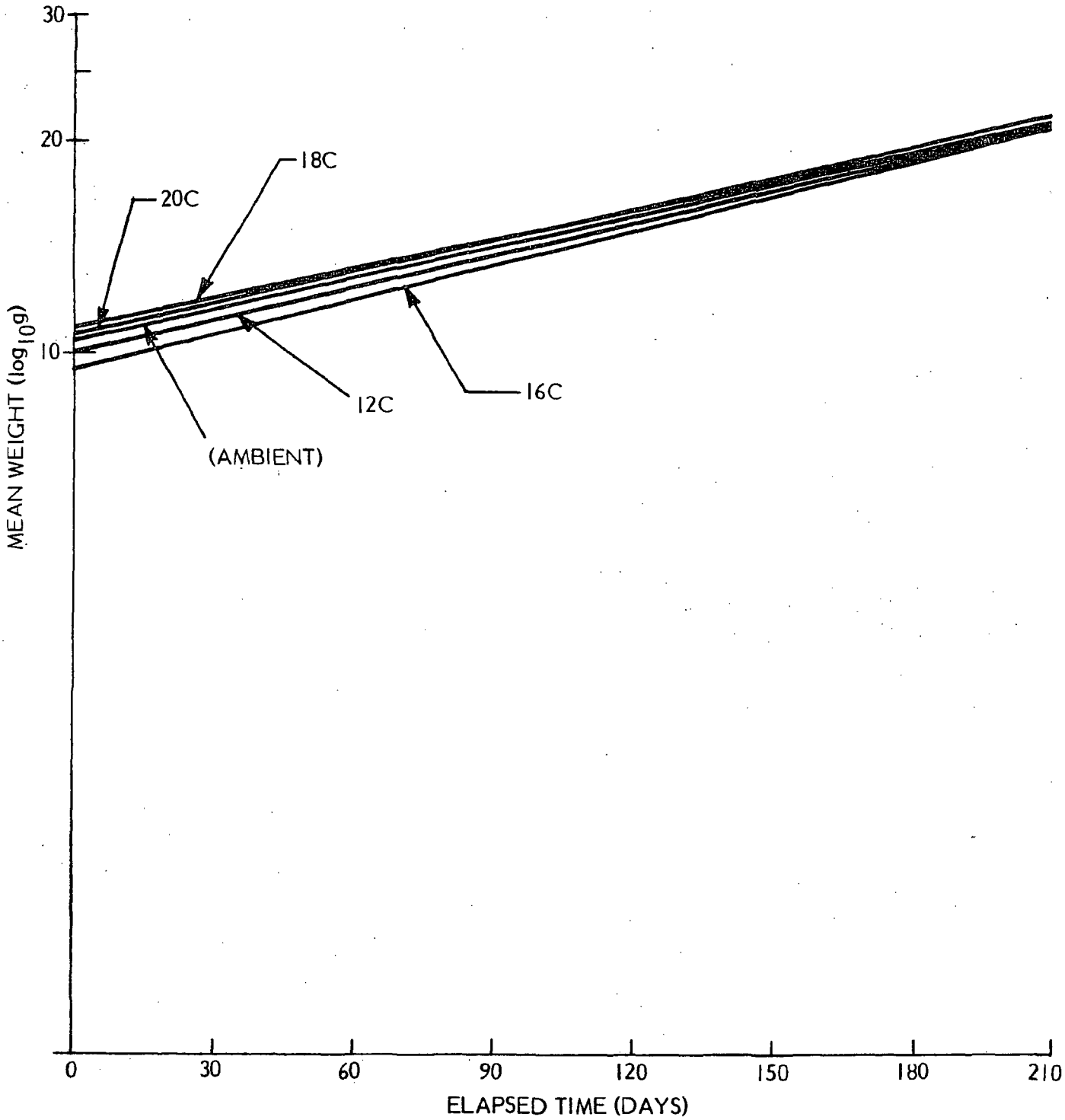


FIGURE 3.1-2

REGRESSION LINES OF TOTAL WEIGHT VERSUS
TIME AT FIVE TEMPERATURES:
BLACK-AND-YELLOW/GOPHER ROCKFISH GROWTH

growth rate (percentage increase in weight per day = % Δ W/day). Growth rates for each experiment were plotted versus temperature in FIGURE 3.1-3.

The black-and-yellow/gopher rockfish grew at rates of from 0.25 to 0.45 percent increase in weight per day. Slowest growth occurred at the highest test temperature, 20 C.

At constant temperatures, black-and-yellow/gopher rockfish grew most rapidly at the surprisingly low temperature of 12 C, with the growth rates decreasing approximately linearly as temperature increased (FIGURE 3.1-3). Since 12 C was the lowest test temperature, it is quite possible that more rapid growth might occur at still colder temperatures.

References to growth rates have, to this point, only been concerned with those test populations held at constant temperatures. In addition to these, the experiment included a test population which was allowed to fluctuate with the ambient seawater temperature. This ambient group had, by far, the highest growth rate (FIGURE 3.1-3) even though the mean temperature (13.2C) was only about 1C above that of the test population with the lowest growth rate (12.1 C). The mechanism by which fluctuating temperatures enhanced the growth of this species is not clear but the resulting increases are readily discernible.

3.1.3 DISCUSSION

Under laboratory conditions of constant temperature and unlimited food supply, maximum growth of juvenile black-and-yellow/gopher rockfish was determined to be less than or equal to 12 C(54 F).

A constant temperature of 20 C (68 F) produced the lowest growth rate. The highest growth rate was achieved in the ambient (fluctuating temperature) treatment which had a mean temperature of 13.2 C (55.8 F).

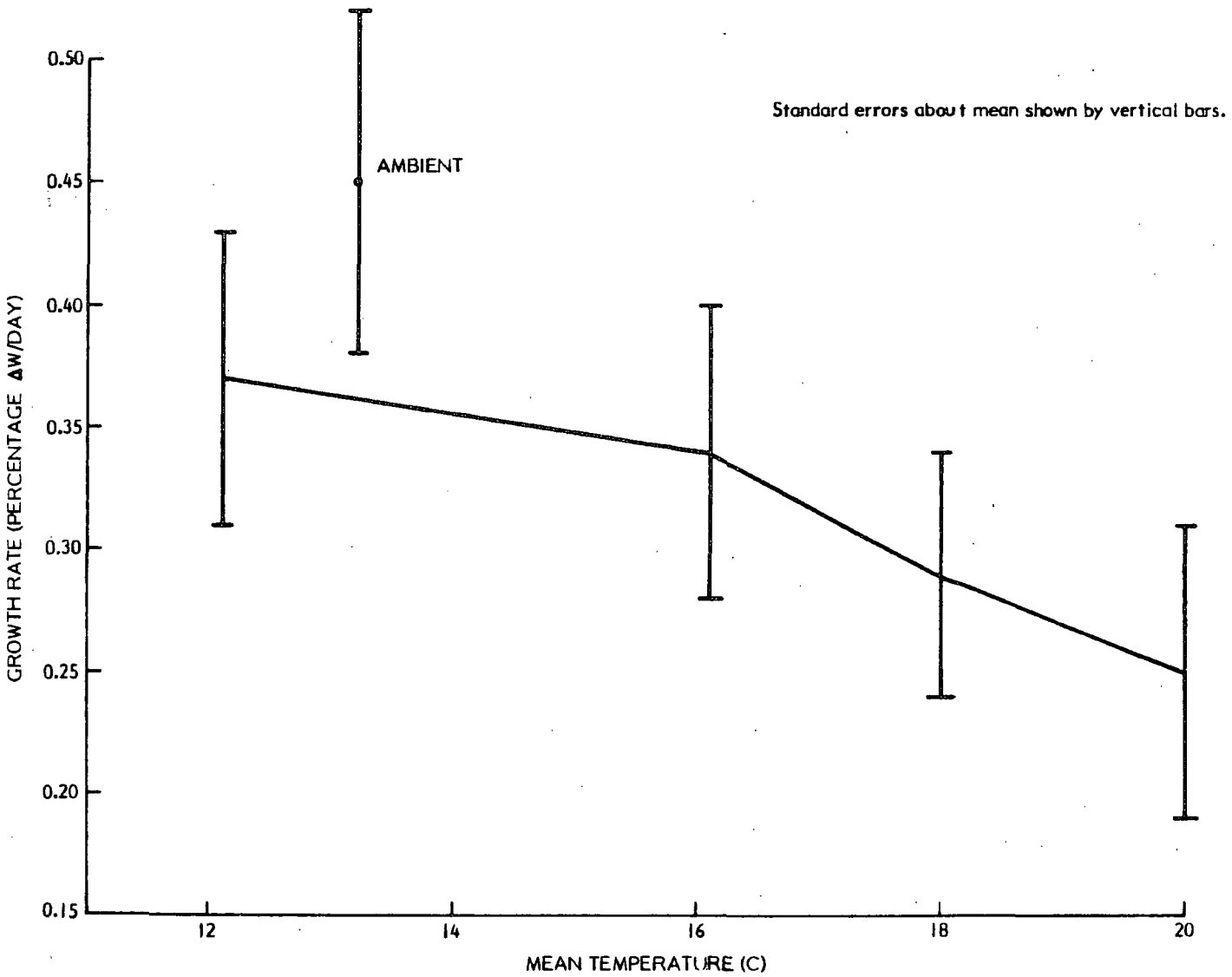


FIGURE 3.1-3

GROWTH RATE VERSUS TEMPERATURE:
BLACK-AND-YELLOW/GOPHER ROCKFISH GROWTH

3.2 BLUE ROCKFISH (SEBASTES MYSTINUS) GROWTH

The results of an experiment to determine the optimum temperature for growth of blue rockfish (Sebastes mystinus) is reported in this section. Blue rockfish were designated a representative important species (RIS) for the Diablo Canyon 316(a) demonstration. The literature on this species was reviewed in a previous report (PGandE 1979b).

The study objective of the optimum temperature for growth experiment on blue rockfish was to determine the temperature or range of temperatures which produce maximum growth under laboratory conditions. The purpose of the experiment was to obtain an estimate of the effects of the power plant thermal plume on long-term growth of blue rockfish juveniles.

3.2.1 METHODS

A summary of the methods used in this experiment is presented below. For a more detailed description of the methods used see APPENDIX B (Procedure 320) and for the experiment protocol see APPENDIX C (experiment 20C).

Juvenile blue rockfish were collected, using a 12 x 12 ft diver-operated lift net, from the area just outside the south breakwater of the Diablo Canyon Power Plant intake cove. All collections for this experiment were made on 20 and 21 June 1979. The fish were transported to the laboratory and held there in 4 x 8 x 2 ft fiberglass tanks maintained at ambient seawater temperature. Just prior to the commencement of the experiment 30 fish were transferred to the experimental 4 x 8 ft tanks, one for each temperature condition. The transfer of experiment animals was followed by a "temperature adjustment" period during which temperatures were raised or lowered at a rate of 1 C per day until the experiment temperature was reached in each tank. Target temperatures were 12, 16, 18, and 20 C with an additional tank maintained at the fluctuating ambient seawater temperature. Once an experiment test temperature was achieved, the fish were allowed to "acclimate" to that temperature for five days. Following this acclimation period, all 30 fish in a test tank were anesthetized

with MS-222 and their individual standard length and wet weight were measured and recorded. These initial measurements marked the start of the experiment (elapsed days: 0) for that particular tank. All test populations were measured between 30 July and 15 August 1979. Measurements were repeated approximately every 30 days for the next 4 months (120 to 121 elapsed days).

All fish were fed an excess ration of frozen brine shrimp (Artemia) daily between 0900 and 1100 for the duration of the experiment. On days when measurements were made, feeding was postponed until after the data were collected. Growth rates were calculated using the formula for exponential growth (Brett et al. 1969): $w = be^{kt}$ (where w=weight, t=time and b and k are constants). When ln w is plotted against t, the slope k x 100 is equal to the growth rate. Tank temperatures were recorded hourly by the laboratory's Autodata Nine data acquisition system. Initially it was necessary to hand transcribe these data for subsequent analysis, but starting in October 1979 the Autodata Nine computer was interfaced with an Alpha Micro computer system allowing the temperature data to be recorded and summarized automatically.

3.2.2 RESULTS

Summary temperature and growth data for the experiment are presented in TABLE 3.2-1. The mean weights of the individual test populations, which were calculated following each observation (approximately every 30 days), are graphically presented in FIGURE 3.2-1. The individual weights recorded for each test population were log transformed, pooled from all observations, and plotted versus time. A least squares linear regression line was calculated for each population from these data. The regression lines are presented in FIGURE 3.2-2. The slope of each regression line, multiplied by 100, is equal to the specific growth rate (percentage increase in weight per day = % Δ W/day). Growth rates for each experiment were plotted against temperature in FIGURE 3.2-3.

Blue rockfish grew at rates ranging from 0.56 to 0.96 percent weight increase per day. The slowest growth occurred at the highest test temperature, 20 C. The blue rockfish held at 20 C began developing lesions after approximately 60

TABLE 3.2-1

SUMMARY OF TEMPERATURE AND GROWTH DATA: BLUE ROCKFISH GROWTH

Target Temperature (C)	Ambient	12	16	18	20
Mean Temperature (C)*	13.9 ± 2.2	12.1 ± 0.3	16.1 ± 0.4	18.0 ± 0.5	20.0 ± 0.7
Start Date	30 July 79	31 July 79	6 August 79	13 August 79	15 August 79
Elapsed Time (Days)	0	0	0	0	0
Mean Standard Length (mm)	60 ± 2	62 ± 2	64 ± 2	68 ± 2	65 ± 1
Mean Wet Weight (g)	6.0 ± 0.5	6.5 ± 0.6	7.1 ± 0.7	8.4 ± 0.8	7.3 ± 0.5
N	30	30	30	29	30
Finish Date	28 November 79	29 November 79	4 December 79	11 December 79	13 November 79
Elapsed Time (Days)	121	121	120	120	90**
Mean Standard Length (mm)	87 ± 3	86 ± 2	87 ± 4	87 ± 4	76 ± 3
Mean Wet Weight	19.9 ± 1.7	18.2 ± 1.7	21.0 ± 2.5	21.1 ± 2.5	12.4 ± 1.5
N	26	28	24	27	19
Growth Rate (% ΔW/Day)	.96 ± .10	.85 ± .11	.88 ± .13	.73 ± .12	.56 ± .16

* Temperature data are ± 2 S.D; all other data are ± 2 S.E.

** 67 percent mortality @ 120 days; no data taken.

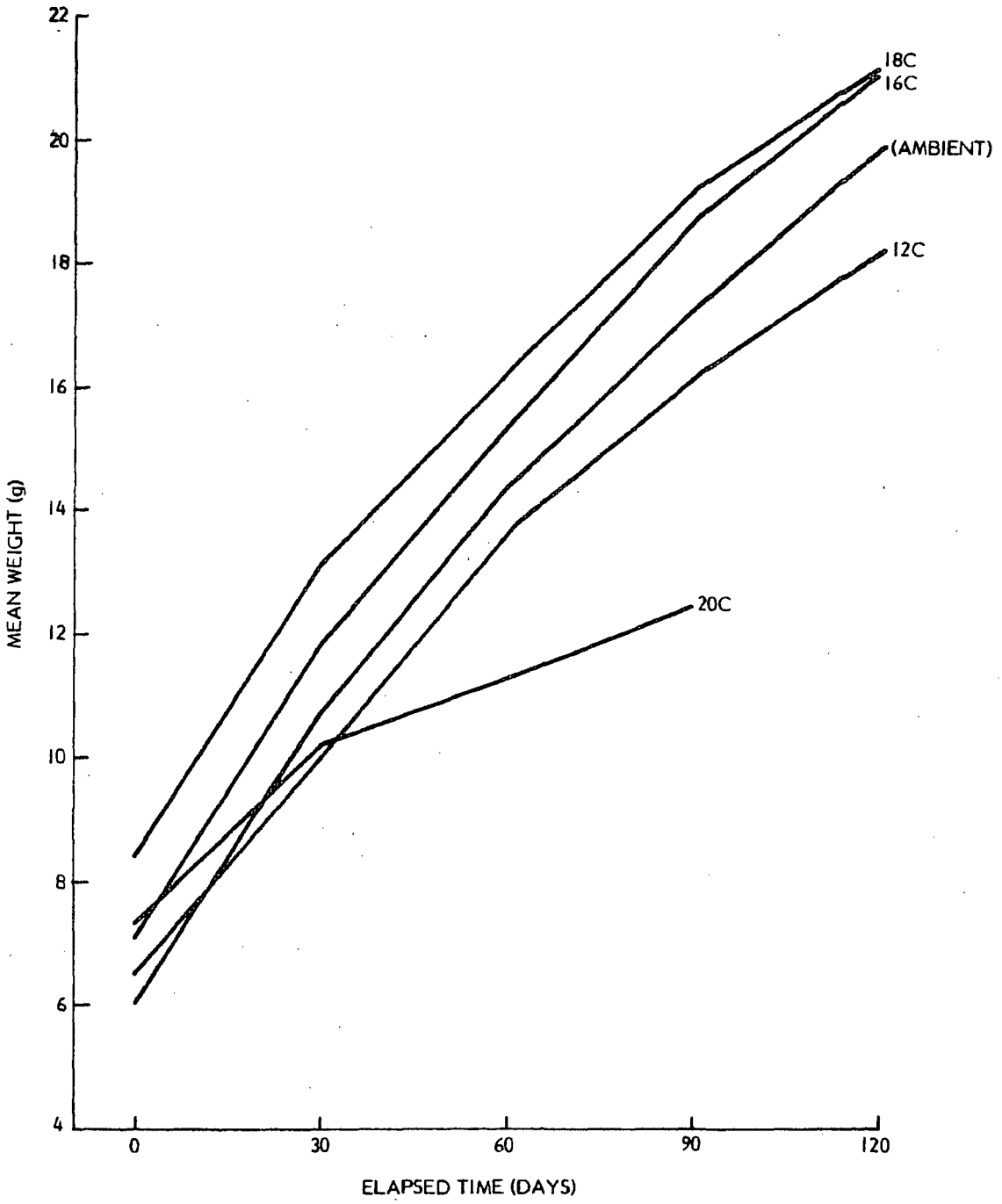


FIGURE 3.2-1

MEAN WEIGHT VERSUS TIME AT FIVE TEMPERATURES:
BLUE ROCKFISH GROWTH

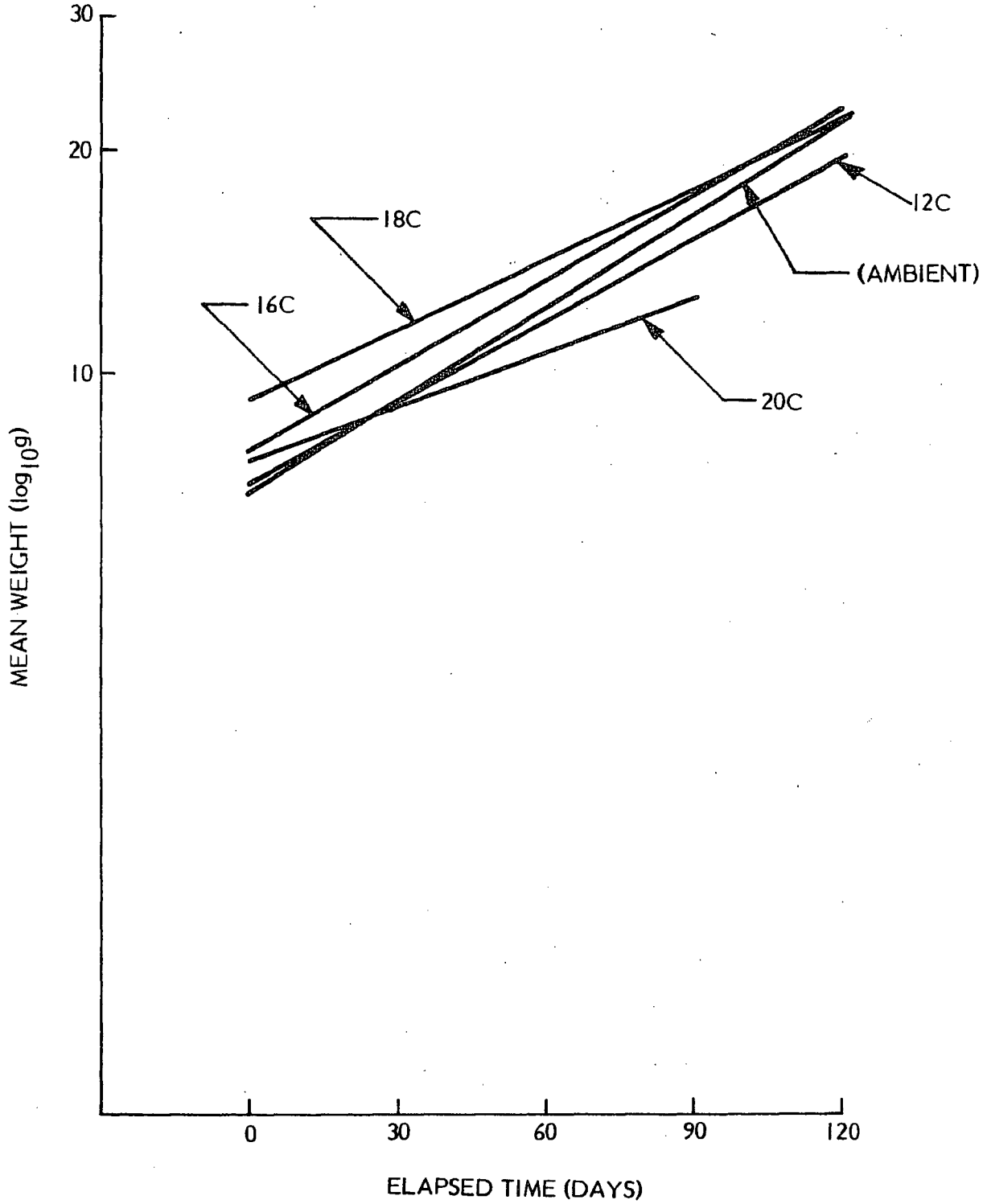


FIGURE 3.2-2

REGRESSION LINES OF TOTAL WEIGHT VERSUS TIME AT FIVE TEMPERATURES: BLUE ROCKFISH GROWTH

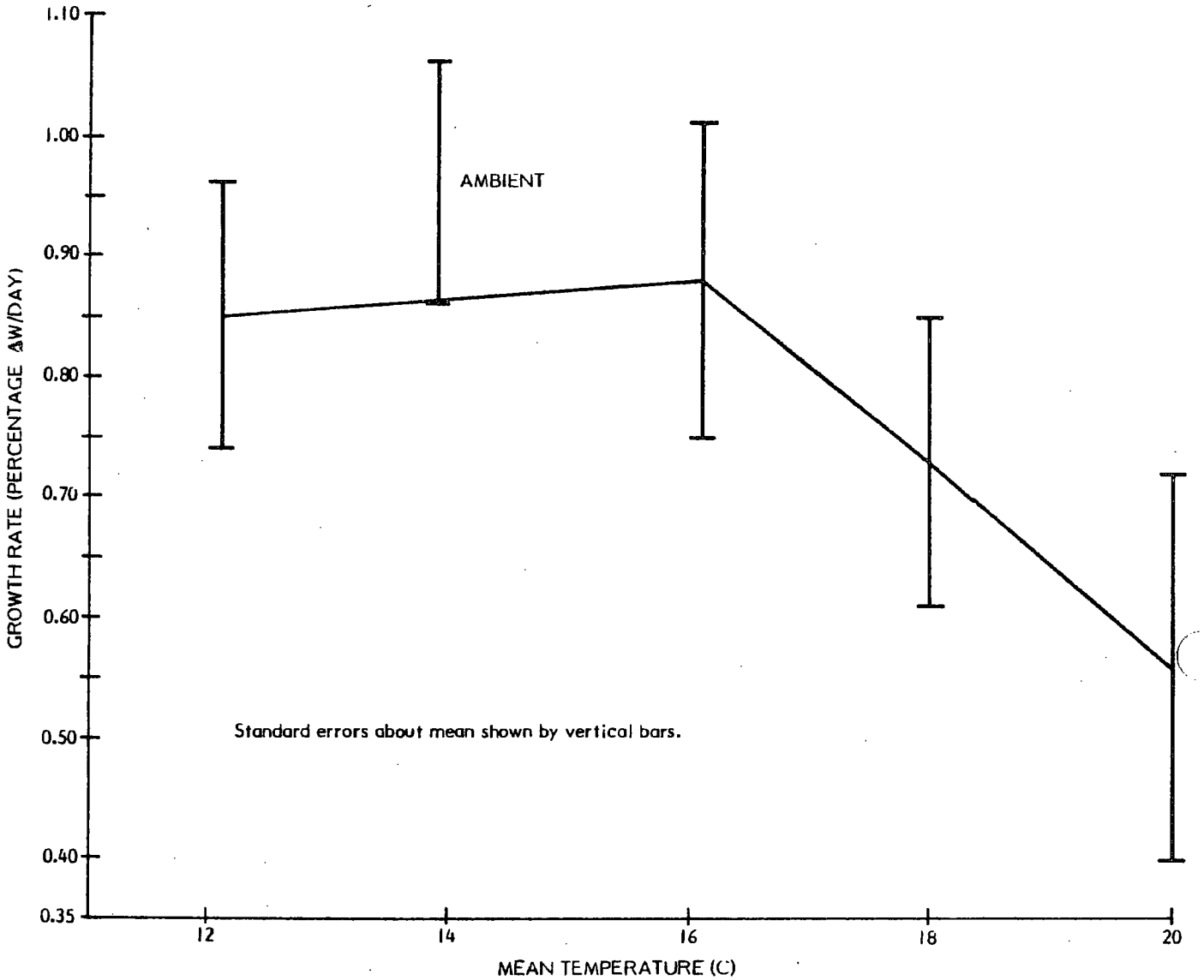


FIGURE 3.2-3

GROWTH RATE VERSUS TEMPERATURE:
BLUE ROCKFISH GROWTH

days of exposure. This condition became more pronounced and, before 120 days had elapsed, 20 of the original 30 fish had died. As a result, no growth measurements were made on this group after 90 days. The 18 C population also began developing lesions just prior to the conclusion of the experiment. The most rapid growth, at a constant temperature, for this species occurred at 16 C (FIGURE 3.2-3), only two degrees below the temperature at which lesions developed. Although these lesions are probably not a direct result of the higher temperatures, it is probable, considering their absence in other species tested at the same time, that the development of the lesions in blue rockfish was enhanced by heat induced stress.

References to growth rates have, to this point, only been concerned with those test populations held at constant temperatures. In addition to these, the experiment included a test population which was allowed to fluctuate with the ambient seawater temperature. This ambient group had the highest growth rate (FIGURE 3.2-3).

The fluctuating temperatures in the ambient tank may have enhanced the growth of blue rockfish, as was apparent for black-and-yellow/gopher rockfish (see Section 3.1).

3.2.3 DISCUSSION

Under laboratory conditions of constant temperature and unlimited food supply, maximum growth of blue rockfish was determined to be between 12 and 16 C (54 and 61 F). A constant temperature of 20 C (68 F) produced the lowest growth rate and contributed to the deaths of 67 percent of the blue rockfish test population. The highest growth rate was achieved in the ambient (fluctuating temperature) treatment which had a mean temperature of 13.9 C (57 F).

3.3 CABEZON (SCORPAENICHTHYS MARMORATUS) EARLY DEVELOPMENT

Two experiments on early development of cabezon (Scorpaenichthys marmoratus) are reported in this section. In the first, the effects of temperature on egg hatching success was investigated. In the second, the effects of temperature on normally hatched larvae was studied.

The cabezon (Scorpaenichthys marmoratus), an RIS for the Diablo Canyon 316(a) demonstration, is the largest representative of the Family Cottidae found off the California coast, reaching a recorded length of 99 cm (39 in.). A common inhabitant of the shallow waters adjacent to the Diablo Canyon Power Plant, this is one of the 15 most abundant fish species observed during the 316(a) subtidal fish observation surveys conducted in Diablo Cove and South Cove (PGandE 1980). During the spawning season, which runs from approximately October through March (O'Connell 1953, Icanberry et al. 1978), cabezon lay their eggs in cohesive masses attached to rocky substrata in relatively shallow water. Egg masses observed in the vicinity of the study area have all been located in water shallower than approximately 7 m (23 ft).

3.3.1 EGG HATCHING SUCCESS

A summary of the literature on the cabezon (Scorpaenichthys marmoratus) was presented by PGandE (1979b). The objective of this experiment was to determine the minimum, optimum and maximum temperatures for normal development of fertilized cabezon eggs by observing hatching success. The purpose was to provide a basis for estimation of possible effects of the power plant discharge on cabezon recruitment in Diablo Cove.

3.3.1.1 METHODS

A summary of the methods used in this experiment is presented below. For a more detailed description of the methods used, see APPENDIX B (Procedure 328) and APPENDIX C (protocol for experiment 28a).

A large cabezon egg mass (weight greater than 1 kg) was collected on 31 January 1980 from the northern portion of Diablo Cove at a depth of 5 m (16 ft). The large cohesive mass of eggs was immediately transferred to the Thermal Effects Laboratory in a bucket of ambient temperature (13.5C) seawater. Upon arrival at the laboratory, the eggs were placed in a larval pot (FIGURE 3.3-1) and maintained in flowing 12C seawater. The eggs were observed carefully during the next three days for any signs of damage incurred during collection. Microscopic examination of samples of the egg mass revealed that it contained embryos at three different stages of development. The different stages were confined to specific areas of the egg mass and most likely represented differing times of deposition and fertilization. Observations made on an egg mass collected prior to this one revealed that eggs located in the interior of thicker portions of the mass developed at a slower rate than eggs at or near the exterior. In an effort to minimize differences between the eggs subjected to each test temperature, all of the eggs for this experiment were taken from a small section of the thin outer margin of the mass. These eggs were examined microscopically to insure that all were healthy and at the same approximate stage of development.

The 1.4-mm-diameter eggs contained a large orange-red yolk sac which occupied approximately 80 percent of each egg's volume. The embryo almost completely encircled the yolk with the posterior 25 to 40 percent of its body free of the yolk sac. The choroid fissure was prominent in the ventral portion of the large eyes. Eye pigment was totally black, not yet reflective. No other pigmentation was evident, with the exception of several small spots located at midbody. A heartbeat was barely discernible. Body movement was observed, but this occurred infrequently.

On 4 February 1980 portions of the egg mass weighing approximately 5 to 6 g were transferred into each of seven different larval pots; each pot was held temporarily at 12C. An additional section of the egg mass was immediately preserved in 4 percent formalin in 50 percent seawater. Egg counts made earlier in the day showed that the density of eggs was approximately 360 per gram. All egg transfers were completed by 3:30 in the afternoon, at which time

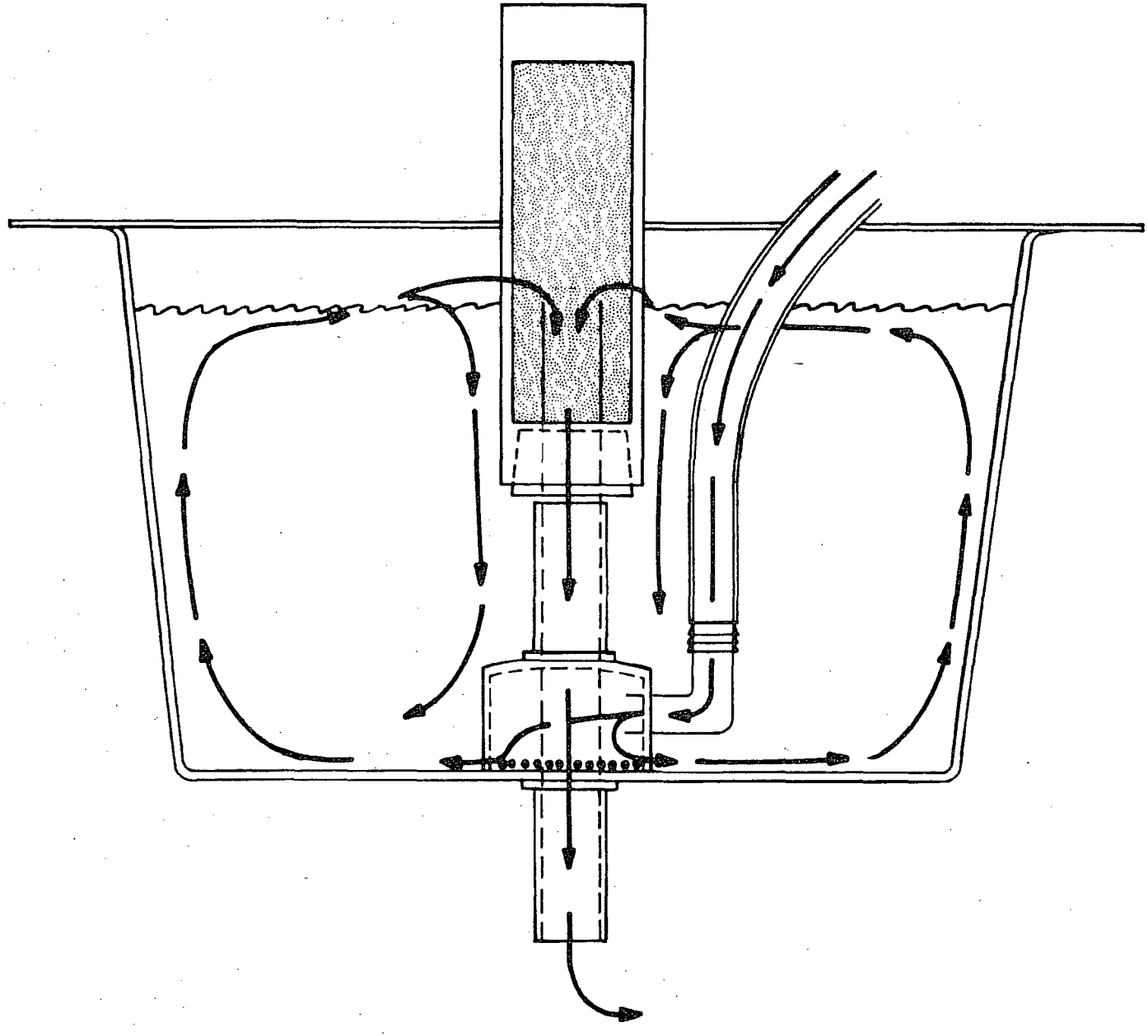


FIGURE 3.3-1

FISH LARVAL CULTURE POT USED FOR CABEZON REARING

temperature adjustment was begun. Target test temperatures were 12, 16, 18, 20, 22, 24, and 26C; all temperatures were achieved within 1 hour. Water flow in the larval pots was maintained at the maximum without dislodging the eggs from the bottom of the pot. Observations were normally made twice daily, once in the morning and again in the afternoon. More frequent observations were made during the actual hatching of the eggs. During each observation the newly hatched larvae were counted and removed from the larval pot; comments were recorded as to the condition of the larvae and the unhatched eggs. The experiment continued at each test temperature until all hatching was completed or it was ascertained that all the remaining eggs were moribund. Temperatures were regulated and hourly temperature data recorded by the Autodata Nine/ Alpha Micro computer system.

3.3.1.2 RESULTS

A summary of temperature and time course of egg hatching data is presented in TABLE 3.3-1 and is shown graphically in FIGURE 3.3-2. Hatching data are presented as both the cumulative number of eggs hatched and the cumulative percentage hatched of the calculated total number of eggs present in each temperature condition. Hatching was complete at 12 and 16C so that the exact number of eggs subjected to those temperatures could be deduced from the number of larvae hatched and counts of the few remaining unhatched eggs.

No hatching was observed at 24 and 26C. Microscopic examination of these eggs revealed that the embryos did not develop beyond the stage they had reached when the experiment commenced. The orange-red yolk in these eggs had leaked out of the yolk sac and filled the interior of the egg in all cases. The once smooth exterior of the egg capsules had taken on a pebbled glass appearance. A white and pink bacterial and/or fungal mat covered many of these eggs.

Those eggs held at 22C also failed to hatch but showed some continuation of development prior to death. Almost all of the embryos had well-developed eyes with highly reflective retinal pigmentation and possible lens formation. The quantity of yolk had diminished considerably in the six days which had elapsed

TABLE 3.3-1

CUMULATIVE NUMBER AND PERCENTAGE EGG HATCH VERSUS TIME:
CABEZON EGG DEVELOPMENT

Start: 04 February 1980 @ 1600 hr

Elapsed Time (hours)	Mean Temperature (C)													
	12.0		16.0		18.0		19.9		21.8*		23.7*		25.6*	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
137							3	0.2	0	0	0	0	0	0
167			78	4.1	168	7.4	41	2.2						
189			289	15.1	186	8.2	52	2.8						
192			1,764	92.0										
208			1,826	95.2	194	8.5	53	2.9						
210	424	22.5												
212	1,627	86.4												
214	1,704	90.4	1,836	95.7										
216	1,725	91.6	1,843	96.1										
232	1,759	93.4	1,846	96.2										
235	1,763	93.6	1,847	96.3										
239			1,854	96.7										
240	1,770	93.9	1,856	96.8										
257	1,802	95.6	1,857	96.8										
259	1,805	95.8												
262	1,812	96.2												
264	1,817	96.4												
284	1,839	97.6	1,858	96.9										
304	1,853	98.4												
312	1,862	98.8												
336	1,873	99.4												
353	1,874	99.5												
384	1,881	99.8												
429	1,882	99.9												

* Observations terminated 10 February 1980.

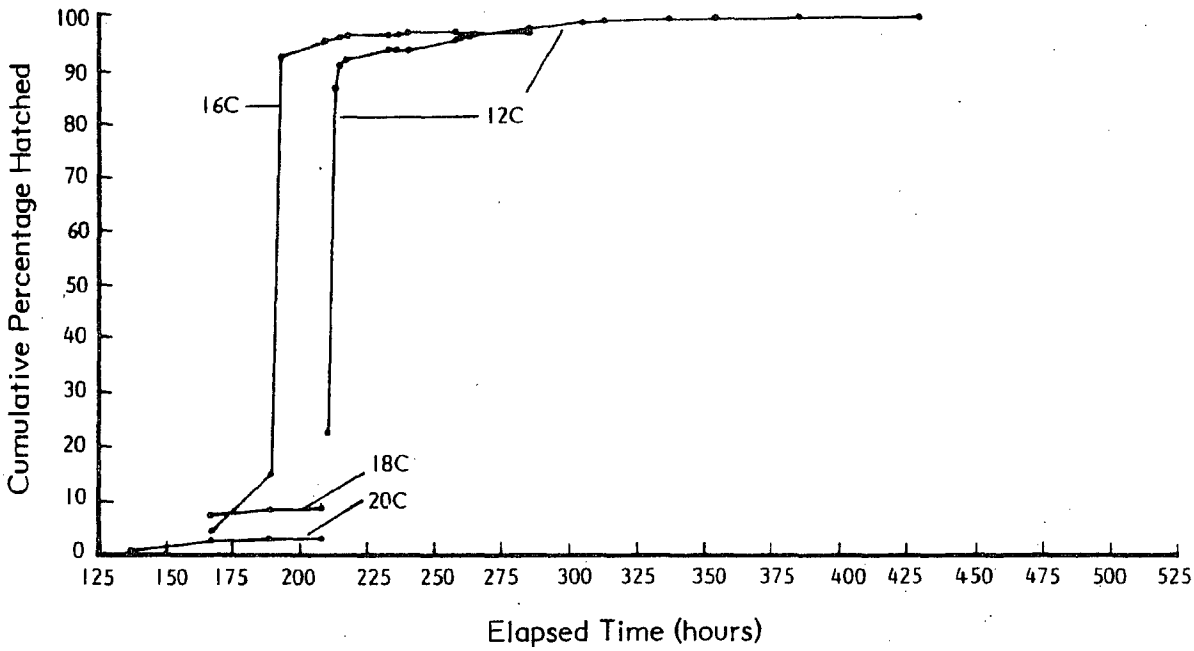


FIGURE 3.3-2

CUMULATIVE PERCENTAGE OF EGGS HATCHED VERSUS TIME:
CABEZON EGG DEVELOPMENT

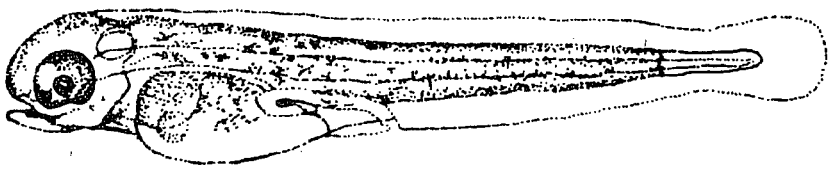
prior to the removal of the eggs from the experiment. The yolk was also retained in the yolk sac after death in all but a few cases. Melanophores were numerous.

A small number of eggs hatched at 18 and 20C (8.5 and 2.9 percent, respectively). Approximately 80 percent of the hatched larvae at 20C had deformed S-shaped bodies. No deformed larvae were observed at 18C. The healthy larvae at both temperatures were quite similar in appearance. The larvae were about 5.5 mm long with a teardrop-shaped yolk sac about 1.5 mm long. Yolk color ranged from amber to green. An oil globule 0.3 mm in diameter was also present within the yolk sac. Numerous melanophores were scattered over the body and several purple pigment spots were located on the head. Iridocytes were fewer in number than the melanophores and were concentrated just above the yolk sac. The eyes were large (0.5 mm) and highly reflective. The transparent pectoral fins were just barely discernible. Heartbeat and circulation were strong and easily observed. Fecal material appeared to be present near the anus. The larvae were strong though sporadic swimmers. They displayed some directional control and the ability to orient to and swim against currents in the larval pot. The 18C larvae appeared to be slightly advanced in their development over those at 20C (slightly more pigment, slightly less yolk, more well developed eyes, etc.). The unhatched dead embryos at both temperatures ranged in their stage of development from embryos unchanged since the start of the experiment (the majority) to larvae trapped in the thin remainder of their egg capsules identical to those which hatched and survived. Eggs in both groups had the pebbled glass appearance described before. Both egg masses were contaminated with the white and pink bacterial and/or fungal mat and with Oscillatoria, a filamentous blue-green alga. Those cabezon eggs held at 12 and 16C experienced almost 100 percent hatching success (99.9 and 96.9 percent, respectively). Newly hatched larvae from these two temperature groups were almost identical. Both had developed further than the 18C larvae (more pigment, more active swimmers). There was, however, a large difference in the quantity of yolk retained by these two groups of larvae. Of all the larvae to hatch, those incubated at 16C retained the least amount of yolk, whereas the larvae incubated at 12C hatched with more yolk than any of the others. The yolk sac at 16C was teardrop-shaped and

contained a quantity of green colored yolk slightly larger than the larva's head. A drawing of a newly hatched cabezon larva (FIGURE 3.3-3), reproduced from O'Connell's "Life History of the Cabezon" (1953), is almost an exact likeness of these 16C larvae. The yolk sac held by a 12C larva was approximately 1.5 times the size found at 16C, was more ovoid in shape, and contained amber colored yolk. Approximately 60 of the eggs incubated at 16C did not hatch. Of these, half had died just prior to hatching, whereas the other half had died at a much earlier stage. Only two eggs did not hatch at 12C; one died early in its development and the other appeared not to have been fertilized. The remainder of the 16C egg mass, as at the higher temperatures, was contaminated with bacteria, fungi, and blue-green algae. The remainder of the 12C egg mass was also contaminated but to a lesser extent.

3.3.1.3 DISCUSSION

Cabezon eggs incubated in the laboratory at 12 and 16C (54 and 61F) hatched with nearly 100 percent success. At 16C the incubation period was reduced and yolk consumption was increased relative to the 12C larvae, but no other differences were observed between the newly hatched larvae in these two groups. Increase in incubation temperature above 16C was detrimental to the developing embryos. The percentages of eggs hatching at 18 and 20C (64 and 68F) were very low (8.5 and 2.9 percent, respectively), and the high incidence of abnormality at 20C makes the long-term survivability of those larvae questionable at best. At temperatures above 20C, no hatching was observed. Exposure to temperatures of 24C (75F) or above resulted in the total cessation of development and rapid death. In the following section, reporting the effects of temperatures on newly hatched cabezon larvae, 50 percent of the animals exposed to 24C were shown to be capable of surviving for more than three days. Thus it would appear that the larvae are able to survive temperatures that would prohibit the development of embryos.



REDRAWN FROM O'CONNELL (1953)

FIGURE 3.3-3

DRAWING OF NEWLY HATCHED CABEZON LARVA

3.3.2 LARVAL HEAT TOLERANCE

The objective of this experiment was to determine the thermal tolerance of newly hatched cabezon larvae. The purpose of the experiment was to provide a basis for estimating the possible effects of the power plant thermal discharge on cabezon recruitment in Diablo Cove.

3.3.2.1 METHODS

A summary of the methods used in this experiment is presented below. For a more detailed account of the methods used, see APPENDIX B (Procedure 327) and APPENDIX C (protocol for experiment 27b).

On 14 November 1979 a cabezon egg mass, approximately 15 cm square, was collected from directly in front of the mouth of Diablo Creek at a depth of 4.5 m (15 ft). The mass was attached to solid rock and articulated coralline algae lining a small depression in the bottom substrate. An adult cabezon was sitting on the eggs and had to be prodded from the site prior to the actual collection. The egg mass was removed by hand and transported to the Thermal Effects Laboratory within a half hour, while being maintained in ambient temperature seawater (14C). In the laboratory, the eggs were placed in a larval pot (FIGURE 3.3-1) and the temperature slowly lowered to 12C, at which it was held constant. The eggs were observed several times daily, with particular attention paid to indicators of an approaching hatch or signs of decay or infection. The transfer of the egg mass from one larval pot to another on 19 November 1979 prompted the hatching of approximately 600 eggs. This hatch was assumed to be premature in view of the large quantity of burgundy colored yolk retained by the larvae and their poor swimming ability compared with that of larvae hatched later. O'Connell's (1953) description of newly hatched cabezon larvae confirmed this assumption of prematurity while accurately describing those larvae which were to hatch later. In spite of the premature nature of the hatch, a thermal tolerance experiment was started on 20 November. The subsequent breakdown of the laboratory boiler unit on 21 November disrupted the laboratory's supply of heated seawater, forcing the experiment to be aborted.

A second hatch estimated at 5,000 to 10,000 larvae occurred between 3 pm on 27 November and 1:30 pm on 28 November. Microscopic examination of the larvae revealed that they matched O'Connell's (1953) description. A drawing of a newly hatched larva, taken from O'Connell's paper, is shown in FIGURE 3.3-3. Mean length of the larvae was 5.7 mm. The teardrop-shaped yolk sac measured 1.6 mm in length, had a maximum depth of 0.5 mm and contained a solitary, spherical oil globule, 0.3 mm in diameter. The color of the yolk varied from amber to chartreuse. Eyes were well developed and darkly pigmented, with reflective retinal pigment just becoming evident. The choroid fissure was still visible. Melanophores were numerous with their highest density being found on dorsal surfaces. Several hundred minute iridocytes were scattered over the body. The larvae were observed to be strong swimmers with a high degree of directional control. Larvae from the earlier, premature hatch had been weak, sporadic swimmers at best.

Beginning at 1:30 pm on 28 November, 100 larvae chosen at random were transferred to each of five larval pots held at 12C; a sixth group of 100 was anesthetized with MX-222 and preserved in 4 percent formalin in 50 percent seawater. Transfer of the larvae was completed by 2:45 pm. Temperatures in the larval pots were gradually brought up to the target temperatures (12, 20, 22, 24, and 26C) by 3:30 pm. Taking the temperature adjustment period into account, 4:00 pm was considered hour 1 of elapsed exposure. All five test populations were observed hourly for the first 6 hours and thereafter observed twice daily (morning and afternoon, at approximately 8:00 am and 3:00 pm). During the observations, dead larvae were counted, removed from the pots, and preserved as described above for later examination. Behavioral notes were made as needed. Lacking a suitable food source, the larvae were not fed during the experiment. The experiment was concluded when 100 percent mortality had occurred in all five larval pots. Test temperatures were regulated and hourly temperature data were recorded by the Autodata Nine/Alpha Micro computer system.

3.3.2.2 RESULTS

Temperature and time course of mortality data are presented in TABLE 3.3-2. Mortality is presented as the cumulative number of dead larvae removed from each temperature condition as time progressed. For three of the five test populations, not all of the 100 original larvae could be accounted for (recoveries ranged from 83 to 100). These discrepancies were most likely due to the rapid disintegration of some larvae following death. A graphic display of the progression of the cumulative percentage mortality with time is given in FIGURE 3.3-4; all percentages were calculated based on the total number of dead larvae recovered from each test population. An inverse relationship can be seen between mortality and temperature. Both the time to initial death and the time to 100 percent mortality decrease with increasing temperature.

Following the experiment, the preserved larvae were examined under a dissecting microscope with particular attention paid to the quantity of yolk retained by each larva at the time of death. A brief synopsis of the findings of these examinations is presented below.

<u>26C Group</u>. The actual mean temperature was 25.8C, time to first death 4 hours, and time to 100 percent mortality 17 hours. These larvae seemed to be identical in appearance to those preserved at the beginning of the experiment (described earlier in this section) with the exception of the contorted positioning of their bodies caused by muscle contractions at the time of death. All of the larvae retained a sizable quantity of yolk. Individual yolk sacs were measured when possible; calculated means for each observation period are presented in TABLE 3.3-3. A direct comparison of these measurements with those taken from the control group is difficult due to the distortion of the yolk sac by the aforementioned muscle contractions. A slight decrease in the quantity of yolk remaining after death was noted between the initial deaths and those occurring later.

<u>24C Group</u>. The actual mean temperature was 24.0C, time to first death 17 hours, and time to 100 percent mortality 113 hours. These larvae differed little

TABLE 3.3-2

SUMMARY OF TEMPERATURE AND MORTALITY DATA:
CABEZON LARVAL HEAT TOLERANCE

Experiment Code: 27b
Experiment Start Date/Time: 28 November, 1979 @ 15:00

Target Temperatures (C): 12, 20, 22, 24, 26
Mean Experiment Temperatures (C): 12.0, 20.2, 21.9, 24.0, 25.8

CUMULATIVE MORTALITY VERSUS TIME

Elapsed Time (hr)	Temperature (C)				
	12	20	22	24	26
4					29
5					66
6					80
17				8	87
24				10	
41			1	13	
48			2	24	
66				39	
72		5		44	
89		15	46	69	
96		29	67	78	
113		78	92	83	
120		92	96		
137		98	100		
144		100			
161	3				
168	4				
185	13				
209	27				
216	64				
234	74				
240	81				
257	83				
264	85				

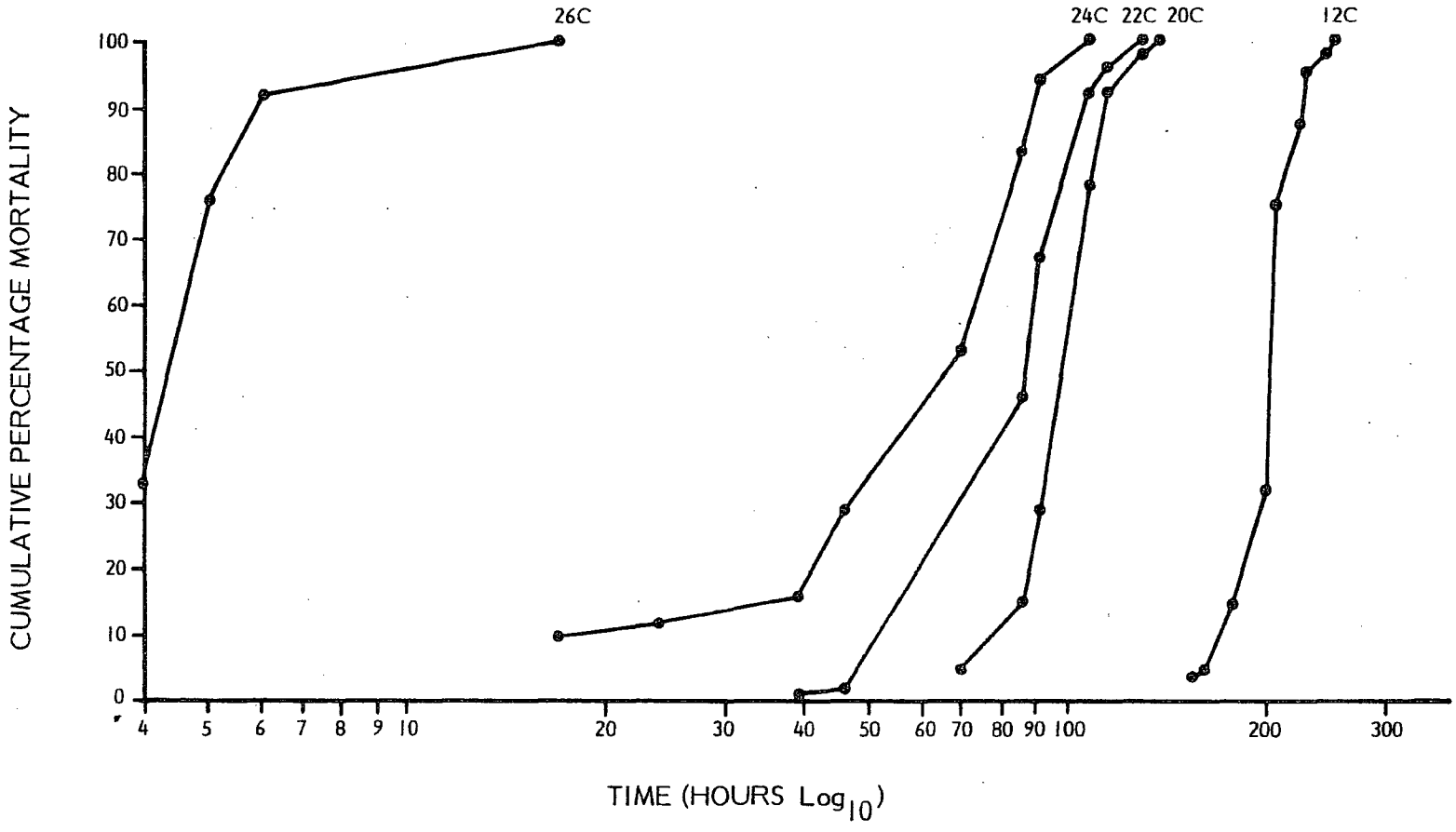


FIGURE 3.3-4

CUMULATIVE PERCENTAGE MORTALITY VERSUS TIME:
CABEZON LARVAL HEAT TOLERANCE

TABLE 3.3-3

MORTALITY AND YOLK SAC DIMENSIONS OF DEAD LARVAE:
CABEZON LARVAL HEAT TOLERANCE

Temper-ature	Time of Exposure (hr)	No. Dead	No. Measured	Yolk-Sac Dimensions (mm) (length X depth)	Comments
12C					
	0	100*	20	1.6 X 0.5	Preserved at outset of exp.
25.8C					
	04	29	10	1.3 X 0.6	
	05	37	10	1.2 X 0.5	
	06	14	10	1.3 X 0.5	
	17	07	06	1.1 X 0.6	01-partially decomposed
24.0C					
	17	08	05	1.1 X 0.4	03-partially decomposed
	24	02	01	0.8 X 0.3	01- " "
	41	03	01	0.6 X 0.5	02- " "
	48	11	07	0.9 X 0.4	03- " "
					01-no yolk
	66	15	09	0.7 X 0.3	06- " "
	72	05	00		05- " "
	89	25	00		20- " "
					05-partially decomposed
	96	09	01	0.5 X 0.3	02- " "
					05-no yolk
					01-oil globule
	113	05	00		04-no yolk
					01-partially decomposed

* Zero hour sample. All larvae alive at time of preservation.

TABLE 3.3-3

MORTALITY AND YOLK SAC DIMENSIONS OF DEAD LARVAE:
CABEZON LARVAL HEAT TOLERANCE
(CONT'D)

Temper-ature	Time of Exposure (hr)	No. Dead	No. Measured	Yolk-Sac Dimensions (mm) (length X depth)	Comments
21.9C					
	41	01	00		01-partially decomposed
	48	01	00		01- " "
	89	44	00		02- " "
					02- " "
					40-no yolk
	97-137	54	00		50-no yolk
					04-partially decomposed
20.2C					
	72	05	00		02-oil globule
					03-no yolk
	89	10	01	0.5 X 0.2	09-no yolk
	96-144	85	00		85-no yolk
12.0C					
	161-264	85	00		85-no yolk

in appearance from those held at 26C. The quantity of yolk retained at death decreased as exposure time increased (TABLE 3.3-3). Of the 44 larvae recovered after 72 hours or more of exposure, only two had any measureable quantity of yolk (one of these retained only its oil globule).

22C Group. The actual mean temperature was 21.9C, time to first death 41 hours, and time to 100 percent mortality 137 hours. The general appearance of these larvae was the same as those preserved at the outset of the experiment. Of all the larvae whose physical condition allowed examination, only two had not totally consumed their allotment of yolk, and those two retained only the oil globule (TABLE 3.3-3).

20C Group. The actual mean temperature was 20.2C, time to first death 72 hours, and time to 100 percent mortality 144 hours. These larvae were similar in appearance to those described previously from the higher temperature groups.

Only three of the 100 larvae recovered retained any food reserves (TABLE 3.3-3).

12C Group. The actual mean temperature was 12.0C, time to first death 161 hours, and time to 100 percent mortality 264 hours. No yolk was retained by any of these larvae. Members of this group were found to have a greater number of melanophores and iridocytes than did larvae from any of the other test populations. Larvae within the group were similar in appearance regardless of the duration of an individual's exposure prior to death.

3.3.2.3 DISCUSSION

The results of this experiment disclosed an inverse relationship between survival time and temperature when newly hatched cabezon larvae, incubated at 12C (53.6F), were subjected to elevated temperatures. At the highest test temperature (25.8C, 78.4F) all deaths appear to have been a direct result of exposure to the elevated temperature, as were the initial deaths (less than 72 hours exposure) at 24.0C (75.2F). This conclusion was based on the post-experiment observation

of sizable food reserves (yolk) retained by these larvae. Those larvae exposed to cooler temperatures, and those that survived for 72 hours or longer at 24.0C (75.2F), were found to have consumed all of their yolk prior to death. In view of the absence of any additional food source, this would indicate starvation to be at least a contributing factor if not the direct cause of death. An increase in the larva's rate of metabolism accompanying increased temperature (Brett 1970) would be expected to accelerate yolk consumption, thereby reducing survival time. Such a reduction can be seen when comparing the results obtained for those larvae held at the apparently sublethal temperatures 12.0, 20.2 and 21.9C (53.6, 68.4 and 71.4F) (TABLE 3.3-3). To obtain direct evidence of this phenomenon, periodic sampling of larvae throughout the experiment would be required.

3.4 ROCK PRICKLEBACK (XIPHISTER MUCOSUS) GROWTH

The results of an experiment to determine the optimum temperature for growth of rock prickleback (Xiphister mucosus) is reported in this section. Rock prickleback was designated a representative important species (RIS) for the Diablo Canyon 316(a) demonstration. A review of the literature on this species was presented by PGandE (1979b).

The study objective of the growth experiment on rock prickleback was to determine the temperature or range of temperatures which produce maximum growth under the prescribed laboratory conditions. The purpose of the experiment was to obtain an estimate of the effects of the power plant thermal plume on long-term growth of rock prickleback juveniles.

3.4.1 METHODS

A summary of the methods used in this experiment is presented below. For a more detailed description of the methods used see APPENDIX B (Procedure 320) and for the experiment protocol see APPENDIX C (experiment 20e).

All of the juveniles used in this experiment were collected in November 1979 from Diablo and "Field's" coves. Collections were made by hand with the aid of small aquarium nets. The majority of the fish were found approximately between the +4.0 and +5.0 ft (MLLW) tidal levels. Intertidal field study stations were avoided during all collections. Forty individuals were haphazardly selected for each of the six experiment temperature conditions. Target temperatures were 12, 14, 16, 18 and 20 C, with a sixth tank allowed to fluctuate with ambient seawater temperature. Because of the small size of these fish (mean total length = 67 mm and mean wet wt = 1.059 g at the start of the experiment), all 40 fish making up each test population were easily accommodated in a 19 liter (5 gal) aquarium. Short sections of ½" PVC pipe (previously leached in seawater) were placed in the aquaria to provide substrate for the secretive and thigmotactic pricklebacks.

Analysis of the stomach contents of rock pricklebacks collected in the vicinity of Diablo Cove showed that algae make up a large percentage of the total volume, while the remainder was comprised of a variety of invertebrates and fish. Due, however, to a lack of information pertaining to the nutritive value and preferability of different algal species, plus difficulties in collecting, handling, and distributing such diverse material as a homogeneous food source, it was decided that the experiment would utilize some other, non-algal diet. Several commercial fish rations, anchovies, squid and frozen brine shrimp were tested as potential foods. In the end, frozen brine shrimp was selected as the experiment diet. Feeding methodology consisted of an excess ration of frozen brine shrimp (Artemia) given daily between 0900 and 1100 for the duration of the experiment. On days when measurements were made, feeding was postponed until after the data were collected.

Growth rates were calculated using the formula for exponential growth (Brett et al. 1969): $w = be^{kt}$ (where w=weight, t=time and b and k are constants). When ln w is plotted against t, the slope k x 100 is equal to the growth rate. Tank temperatures were recorded hourly by the Thermal Effects Laboratory's Autodata Nine data acquisition system. Initially it was necessary to hand transcribe these data for subsequent analysis, but starting in October 1979 the Autodata Nine computer was interfaced with an Alpha Micro computer system allowing the temperature data to be recorded and summarized automatically.

Aquaria containing the test populations were raised or lowered to the appropriate test temperature at a rate of 1 C/day. After a period of "temperature adjustment" the fish were anesthetized with MS-222 and measured. Total lengths were measured to the nearest millimeter and weights taken to the nearest milligram using a Mettler H35AR balance (excess water was blotted from the fish prior to weighing). Subsequent size measurements were made approximately every 30 days for the three month duration of the experiment (90 to 91 elapsed days). All fish were initially measured between 10 and 18 December 1979. The methods used for recording and analyzing temperature and size data were the same as in past experiments.

3.4.2 RESULTS

Temperature and growth data are presented in TABLE 3.4-1. Because of the number of measurements taken in these experiments, and in an effort to most clearly show the resultant growth of the entire experiment period, only growth data (mean length and weight) recorded at the beginning and end of the experiment are presented in this table. The mean weights of the individual test populations, which were calculated following each observation (approximately every 30 days), are, however, graphically presented in FIGURE 3.4-1. The individual weights recorded for each test population were log transformed, pooled from all observations, and plotted versus time. A least squares linear regression line was calculated for each population from these data. The regression lines are presented in FIGURE 3.4-2. The slope of each regression line, multiplied by 100, is equal to the specific growth rate (percentage increase in weight per day = $\% \Delta W$/day). Growth rates for each experiment were plotted against temperature in FIGURE 3.4-3.

The rock prickleback had a relatively high growth rate ranging from 0.88 to 1.42 percent weight increase per day. Slowest growth occurred at the highest test temperature, 20 C.

The growth rates for the rock pricklebacks (FIGURE 3.4-3) exhibit an anomalously low value for the 16 C population (0.97 percent Δ W/day) flanked by much higher values for the 14 C and 18 C groups (1.25 and 1.28 percent ΔW/day, respectively). Inspection of FIGURE 3.4-1 reveals that growth at 16 C paralleled that at 14 and 18 C for the first 30 days of the experiment, then nearly leveled off between 30 and 60 days. Between 60 and 90 days, the 16 C group again produced growth comparable to the 14 and 18 C groups (20 percent weight increase at 14 C, 18 percent at 16 C, and 21 percent at 18 C). Because the decreased growth rate of the 16 C group between 30 and 60 days did not continue or decrease further, but instead increased between 60 and 90 days, it seems highly unlikely that the initial decrease and subsequent low growth rate for the entire experiment period resulted from the temperature to which the fish were subjected. It would then appear, from the growth rates of the other test

TABLE 3.4-1

SUMMARY OF TEMPERATURE AND GROWTH DATA: ROCK PRICKLEBACK GROWTH

Target Temperature (C)	Ambient	12	14	16	18	20
Mean Temperature (C)*	14.1 ± 1.4	12.0 ± .2	14.1 ± .3	16.0 ± .2	18.0 ± .1	20.0 ± .2
Start Date	10 December 79	11 December 79	12 December 79	14 December 79	17 December 79	18 December 79
Elapsed Time (Days)	0	0	0	0	0	0
Mean Total Length (mm)	66 ± 2	65 ± 2	68 ± 3	65 ± 2	68 ± 2	68 ± 2
Mean Wet Weight (g)	.924 ± .089	.952 ± .102	1.097 ± .137	1.046 ± 1.07	1.141 ± .132	1.194 ± .101
N	40	40	40	40	40	40
Fish Date	10 March 80	10 March 80	11 March 80	13 March 80	17 March 80	17 March 80
Elapsed Time (Days)	91	90	90	90	91	90
Mean Total Length (mm)	98 ± 3	91 ± 4	99 ± 4	89 ± 3	96 ± 3	84 ± 3
Mean Wet Weight	3.495 ± .310	2.766 ± .379	3.462 ± .359	2.647 ± .250	3.595 ± .342	2.394 ± .274
N	37	34	40	40	39	19
Growth Rate (%ΔW/Day)	1.42 ± .14	1.16 ± .19	1.25 ± .17	0.97 ± .15	1.28 ± .16	0.83 ± .15

*Temperature data are ± 2 S.D; all other data are ± 2 S.E.

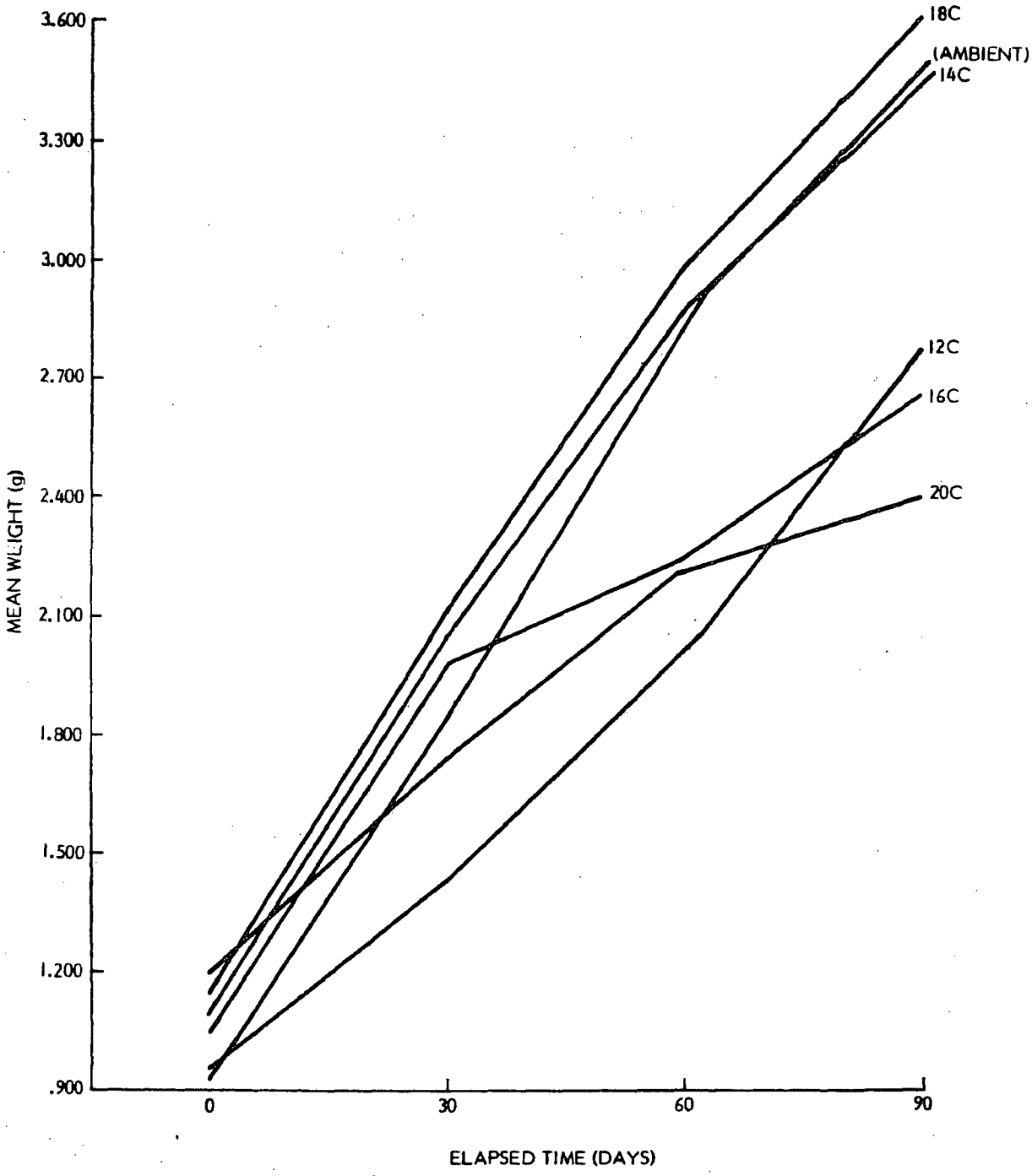


FIGURE 3.4-1

MEAN WEIGHT VERSUS TIME AT SIX TEMPERATURES:
ROCK PRICKLEBACK GROWTH

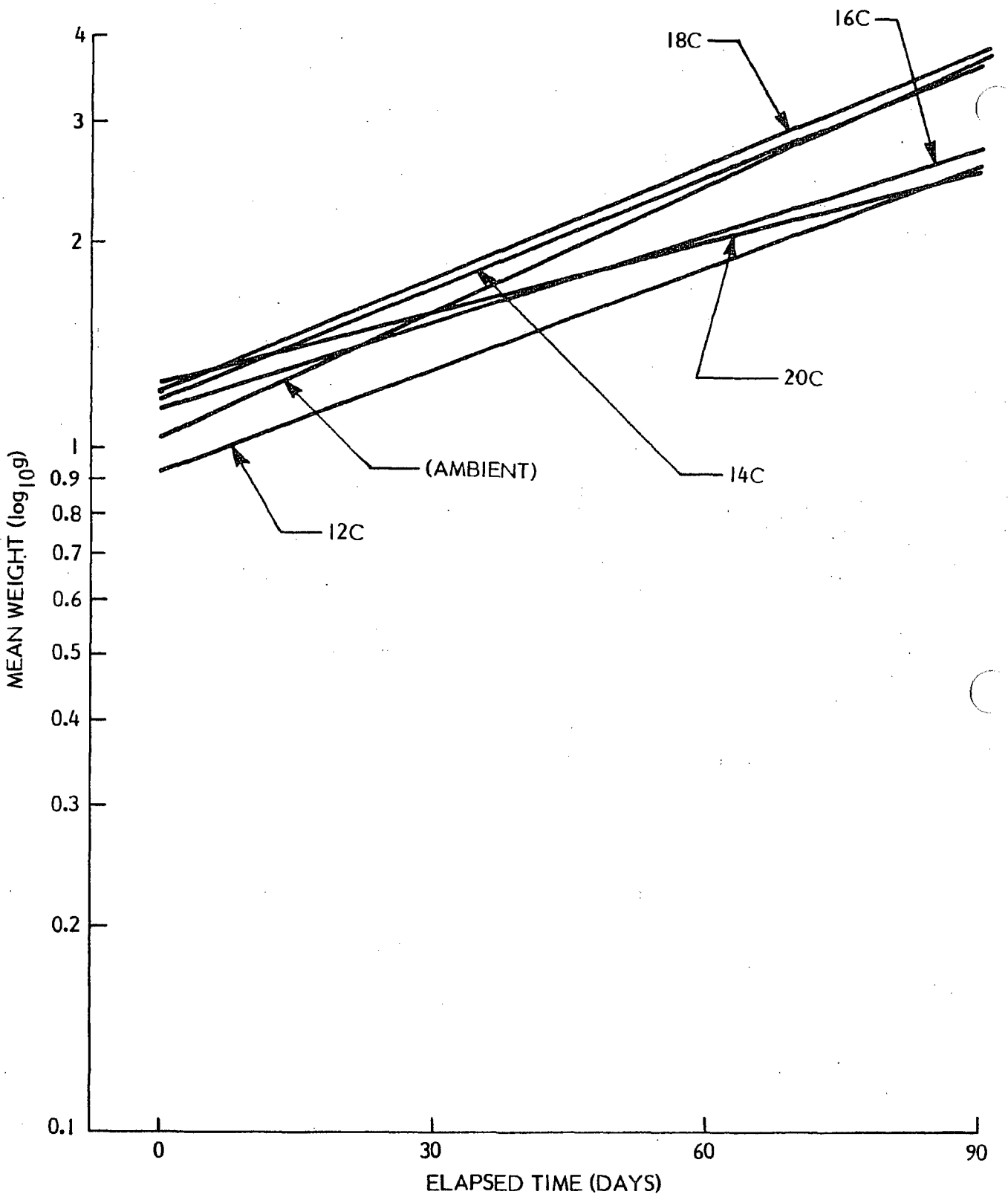


FIGURE 3.4-2

REGRESSION LINES OF TOTAL WEIGHT VERSUS TIME AT SIX TEMPERATURES: ROCK PRICKLEBACK GROWTH

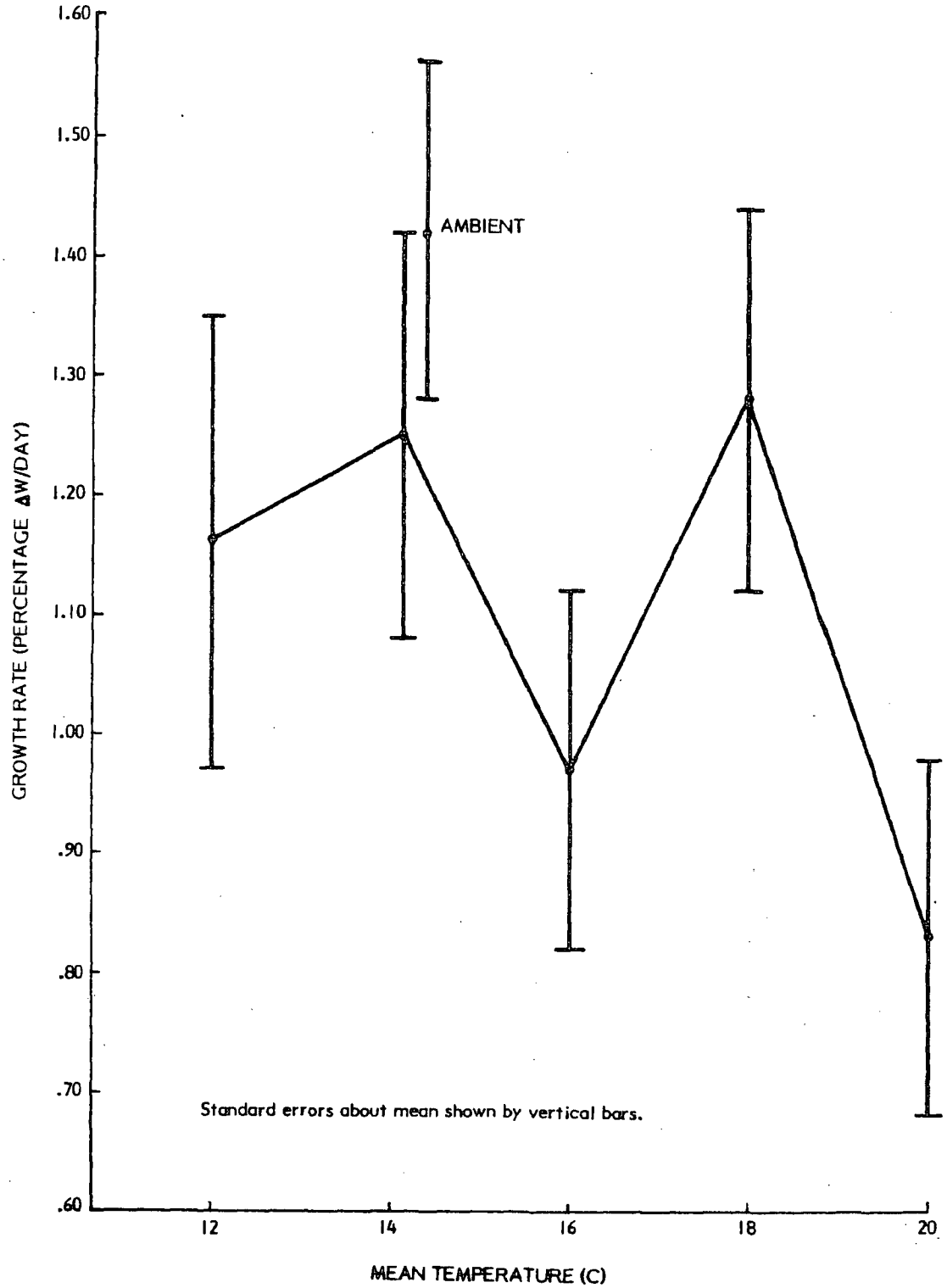


FIGURE 3.4-3

GROWTH RATE VERSUS TEMPERATURE:
ROCK PRICKLEBACK GROWTH

populations, that the optimum temperature for growth (at constant temperatures) is between 14 C and approximately 18 C and may, in fact, be near 16 C.

References to growth rates have, to this point, only been concerned with those test populations held at constant temperatures. In addition to these one test population was held in ambient seawater with a fluctuating temperature. The ambient group had, by far, the highest growth rate (FIGURE 3.4-3). The mean temperature of the ambient group was 14.1 C. The mechanisms by which fluctuating temperatures enhanced the growth of rock prickleback are not clear but the resulting increases are readily discernible.

3.4.3 DISCUSSION

Under laboratory conditions of constant temperature and unlimited food supply, maximum growth of rock prickleback occurred between 14 and 18 C (57 and 64 F). A constant temperature of 20 C (68 F) produced the lowest growth rate. The highest growth rate occurred in the ambient (fluctuating temperature) treatment ($\bar{X}$ = 14.1 C or 57.4 F).

3.5 BLACK ABALONE (HALIOTIS CRACHERODII)

Two experiments on black abalone (Haliotis cracherodii) are reported in this section. The effects of temperature on early development are reported in an experiment on larvae raised in the laboratory. In the second experiment, the effects of temperature and diet were investigated using juvenile animals.

The black abalone (Haliotis cracherodii) is an RIS for the Diablo Canyon 316(a) demonstration. This species, distributed primarily in the intertidal region but also occurring in the shallow subtidal region, is one of the most numerous and conspicuous of intertidal invertebrates in Diablo Cove (Burge and Schultz 1973). The black abalone is harvested commercially and by sportfishermen. The literature on this species was reviewed by PGandE (1979b).

3.5.1 LARVAL DEVELOPMENT

A series of experiments was conducted to investigate the effects of temperature on development of larvae in the black abalone. The purpose was to provide information on the possible impact of the power plant thermal effluent on survival of the larvae. The objectives were to determine the minimum, optimum and maximum temperature ranges for normal development and to measure the effect of temperature on developmental rates.

A large body of literature exists on the general pattern of development in the Haliotidae (Ino 1952, Crofts 1937, Leighton 1972, 1974, Murayama 1935). Although development in other California species has been previously investigated, the black abalone has heretofore been neglected in the literature. This report will be the first account of development in this species. Comparisons with published reports on the other California species and a brief account of the development pattern will be made.

3.5.1.1 METHODS

A summary of the methods employed in the investigations into the effects of temperature on development in black abalone is presented below. For a more detailed account of the laboratory procedures see APPENDIX B (Procedure 307) and protocol, APPENDIX C (experiment 7c).

Pre-experimental

Adult black abalone were collected from areas surrounding Diablo Canyon. Animals were held for a period of at least two weeks in the laboratory at ambient seawater temperature. During this period the animals were fed Macrocystis to excess.

Spawning of the black abalone in nature is reported to occur in the late spring and fall (Leighton and Boolootian 1963). Attempts at artificially inducing spawning would presumably have a greater probability of success if spawning stock was harvested and spawned during this period and would require adult abalone with plump, gravid gonads. Black abalone are not responsive or are only marginally responsive to spawning techniques currently used for other haliotid species in mariculture operations. After considerable difficulty a method was devised which provided good results in males and marginal results in females. A period of approximately 18 hours of dessication followed by emergence in seawater at 18 C was used. U.V. irradiated seawater was added with a flow of 300 ml/min for up to 12 hours.

Experimental

The incubation gradient apparatus used in this experiment was described by PGandE (1977). It consists of a large (3 by 4 ft by 8 in.) water bath which is heated and cooled at diagonal corners giving rise to a thermal gradient. The water bath is compartmentalized, aerated and insulated to maintain constant temperatures once thermal stability is achieved. Test organisms are placed into beakers which can be located in compartments at any desired test temperatures

across the established gradient range. Thermistors are supplied to each compartment which provide input to the Autodata Nine computer for continuous temperature recording.

Following spawning, the sperm and eggs were collected and the eggs fertilized. The fertilized eggs were then washed repeatedly to remove any excess sperm which could act as a substrate for bacterial infection. The eggs were then placed in a large flow-through seawater system at ambient temperature. A known volume of the seawater containing approximately 200,000 embryos was transferred to a sterile 2-liter beaker. The beaker was then placed in the appropriate chamber in the incubation gradient with gentle aeration. Six experimental temperatures were used for this prehatch cohort: 8, 11, 14, 17, 21, and 23 C. An ambient control group was also maintained.

Observations on the six experimental treatments were made every 4 to 8 hours prior to hatching. Following hatching, observations were taken approximately daily. Experimental observations consisted of a sample of approximately 100 larvae removed by pipette and placed in a gridded culture dish for observation. Observations included number of larvae at each developmental stage, number of mortalities and number and type of abnormalities, if any. Development stages as described by Ino (1952) and Leighton (1972) were used in the staging of the larvae.

After the ambient control group hatched, another six experimental groups were established (posthatch cohort). Temperatures used in this posthatch cohort test were 9, 13, 15.5, 18, 20 and 23 C. Observations of the posthatch cohort followed the same procedure as described for the prehatch cohort.

Water was changed in the experimental beakers daily using 0.2 m-filtered seawater adjusted to the experimental temperature. Antibiotics (penicillin-G and streptomycin-sulfate) were added at this time to a final concentration of 150 ppm, to control bacterial growth. Water quality consisting of pH and salinity measurements were taken once during the experiment. This allowed a check to to be made for any extraneous sources of experimental variation. Water

temperatures were recorded hourly by a thermistor probe located in each beaker, connected to the Autodata Nine computer.

3.5.1.2 RESULTS

Summarized below are the results of the temperature, water quality, mortality and development measurements and observations.

Temperature

Summarized temperature data from the incubation gradient are presented in TABLE 3.5-1. Means and standard deviations are given for the time course of the experiment. Mean temperatures ranged from 8.0 to 23.9 C.

Water Quality

Water quality measurements taken during the course of the experiment are presented in TABLE 3.5-2. Water quality measurements taken from the incubation gradient after one day of treatment do not differ appreciably from those taken from a fresh sample of 0.2 μm filtered seawater. From these data we may infer that the experimental treatments had no appreciable effect on salinity and pH and did not constitute an extraneous source of variation within the experiment.

Mortality

Mortality was measured in each of the samples as the proportion of larvae undergoing abnormal development within a treatment. This index was used because direct determination of mortality proved to be technically unfeasible, first, because no completely acceptable criteria are known by which a dead larva can be identified (periodic cessation of swimming was observed in apparently normal larvae). Secondly, because non-swimming larvae were observed to sink, indicating that dead larvae would accumulate at the bottom of the experimental chamber, making representative sampling from continuously maintained cultures

TABLE 3.5-1

SUMMARY TEMPERATURE DATA
FROM THE INCUBATION GRADIENT:
BLACK ABALONE LARVAL DEVELOPMENT

Experiment conducted from 1100, 11-28-78 to 1300, 12-03-78.

Chamber	Mean Temperature (C)	Standard Deviation	Target Temperature (C)[a]
91	6.2	0.2	
92	8.0	0.3	8.0
93	9.1	0.6	
94	10.2	0.7	11.0
95	12.3	0.8	
96	13.5	0.7	
97	14.2	0.6	14.5
98	14.9	0.6	
99	15.4	0.6	
100	16.1	0.6	
101	17.0	0.7	17.0
102	18.0	0.8	
103	19.1	0.8	
104	20.8	0.6	
105	20.9	0.5	
106	21.2	0.5	21.0
107	22.2	0.3	
108	23.1	0.4	
109	23.9	0.5	23.3
110	25.1	0.6	

a Target temperatures indicated only for experimental chambers.

TABLE 3.5-2

WATER QUALITY MEASUREMENTS FROM INCUBATION GRADIENT TAKEN ON 30 NOVEMBER 1978: BLACK ABALONE LARVAL DEVELOPMENT

Measurements on 0.2 μ m filtered ambient seawater are also included for comparison.

Water Source	pH	Salinity (ppt)
0.2 μ m ambient seawater	8.17 @ 20.9 C	33
23.3 C treatment	8.21 @ 20.9 C	34
21.0 C treatment	8.05 @ 21.0 C	34
17.0 C treatment	8.18 @ 20.5 C	33
14.5 C treatment	8.16 @ 20.4 C	34
11.0 C treatment	8.13 @ 20.5 C	32
8.0 C treatment	8.13 @ 20.8 C	32

impossible without introducing mechnical damage as a likely factor in mortality of the living larvae. Abnormal larvae were identified by grossly different morphology or activity from those of normally developed abalone larvae as described by Crofts (1937), Ino (1952), Leighton (1974) and Morse et al. (1979b). A summary of the numbers of abnormal larvae and noncleaved embryos observed in the course of the experiment is presented in TABLE 3.5-3. The proportion of noncleaved embryos at the initial observation and its 95 percent confidence interval were calculated for each treatment (TABLE 3.5-4). Inspection of these values reveals comparability between treatments, lending support to the assumption of a homogeneous population used to initiate the experiment.

The proportions of abnormal larvae observed were adjusted to allow for the number of noncleaved embryos (TABLE 3.5-5). In the early observations (3 and 10 hours) the most commonly seen abnormalities at the higher temperatures were blebs associated with unequal cell division. In the later observations, lysis of the cellular contents was the most commonly noted abnormality. In many cases lysis may have been confused with advanced stages of bacterial infection. Lysis was not only restricted to the higher temperature treatments. At the 20-hour observation of the 8 C treatment, lysis accounted for over half of the large number of abnormalities noted. Because in almost all cases such gross abnormalities would lead to death, they are considered to represent mortalities even though the larvae were often still alive at the time of observation. The percentages of mortality in the treatments are presented in FIGURE 3.5-1.

Comparisons of the sample proportions were carried out using a method described by Fleiss (1973). Chi-square values reflecting levels of statistical significance were obtained at each observation period for all temperatures. A summary of the statistical analyses at each observation and a posteriori groupings of the treatments is given in TABLE 3.5-6. As is revealed by FIGURE 3.5-1 and TABLE 3.5-6 differences in proportion of mortality between temperatures increase with time. This is evidenced by the increase in the overall x^2 value with time and the fact that at 3 hours no significant difference between treatments was found. By the 28-hour observation (D in TABLE 3.5-6) significant levels of mortality were restricted to the two highest temperature

TABLE 3.5-3

SUMMARY OF ABNORMAL LARVAE
AND NONCLEAVED EMBRYOS OBSERVED:
BLACK ABALONE LARVAL DEVELOPMENT

Temperature (C)	Observation Time (hours)	Sample Size	No. Abnormal	No. Non-cleaved
	3	75	14	18
	10	—	—	—
8	20	130	89	0
	28	78	34	18
	40	135	86	33
	3	73	12	22
	10	84	21	20
11	20	86	31	0
	28	120	24	24
	40	229	60	52
	3	71	12	18
	10	76	12	20
14.5	20	73	36	0
	28	114	26	24
	40	131	21	5
	3	72	16	14
	10	85	13	24
17	20	79	41	0
	28	84	28	21
	40	93	44	23
	3	81	14	20
	10	75	10	23
21	20	58	34	0
	28	92	37	40
	40	71	50	21
	3	73	13	21
	10	87	58	22
23	20	169	152	8
	28	52	37	14
	40	43	43	0

TABLE 3.5-4

PROPORTIONS OF LARVAE AT THE THREE HOUR OBSERVATION EXHIBITING NO CLEAVAGE: BLACK ABALONE LARVAL DEVELOPMENT

Temperature (C)	Proportion	95 Percent Confidence Level[a]
8	0.240	0.125 to 0.355
11	0.301	0.184 to 0.418
14.5	0.253	0.134 to 0.372
17	0.194	0.076 to 0.312
21	0.247	0.136 to 0.358
23	0.288	0.171 to 0.405

[a] $p \pm \frac{1}{\sqrt{n}}$

TABLE 3.5-5

PROPORTION OF LARVAE WITH ABNORMALITIES AT 3, 10, 20, 27 AND 40 HOURS: BLACK ABALONE LARVAL DEVELOPMENT

Temperature (C)	Observation (h)				
	3	10	20	27	40
8	0.246	--	0.685	0.567	0.843
11	0.235	0.328	0.360	0.250	0.339
14.5	0.226	0.214	0.493	0.289	0.167
17	0.276	0.213	0.519	0.444	0.629
21	0.229	0.192	0.586	0.712	1.000
23	0.250	0.892	0.944	0.934	1.000

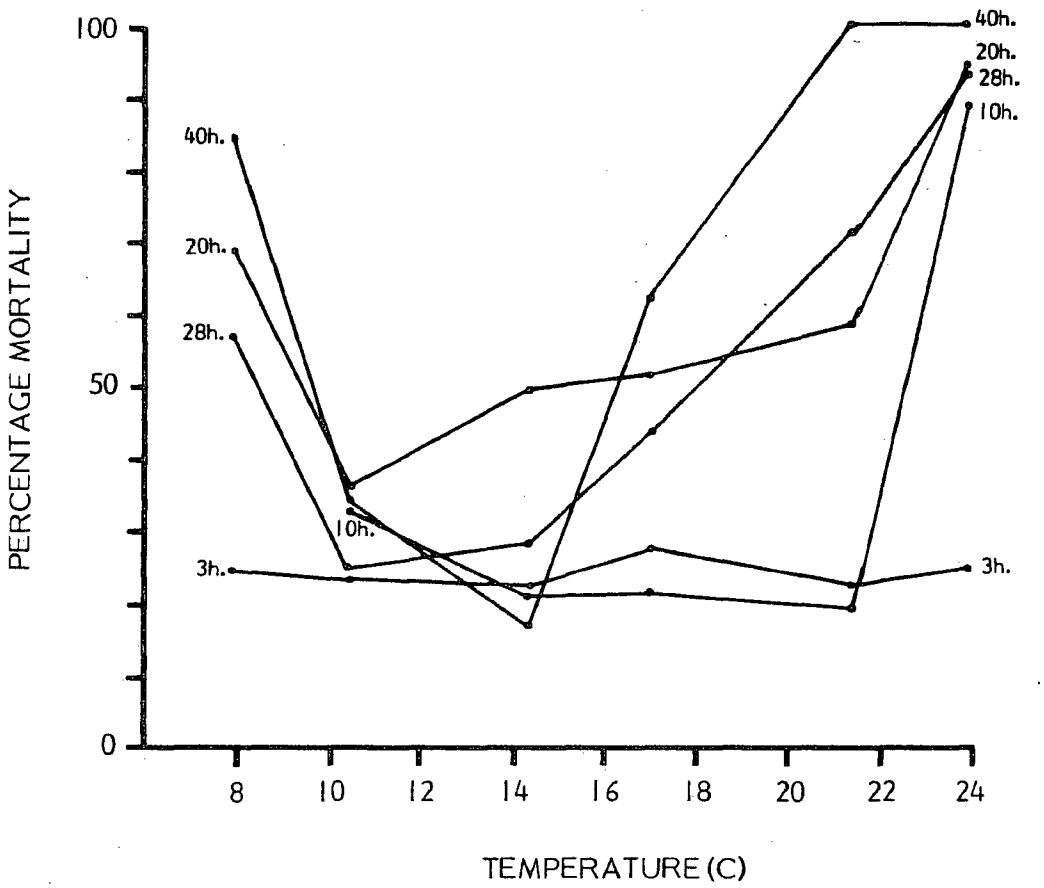


FIGURE 3.5-1

PERCENTAGE MORTALITY VERSUS
TEMPERATURE AT FIVE OBSERVATIONS:
BLACK ABALONE LARVAL DEVELOPMENT

TABLE 3.5-6

SUMMARY OF STATISTICS ON MORTALITY LEVELS AT 3, 10, 20, 27 AND 40 HOURS: BLACK ABALONE LARVAL DEVELOPMENT

Source	d.f.	x^2	$x^2_{\infty} = .01$	
A. 3 hour observation				
Overall: 8, 11, 14, 17, 21, 23 C	5	0.523	15.086	n.s.*
B. 10 hour observation (8C not included; no observation				
Overall: 11, 14, 17, 21, 23C	4	94.343	13.277	s.**
Group 1: 11, 14, 17, 21C	3***	2.9106	11.345	n.s.
Difference: 23C - 11 + 14 + 17 + 21C	4	91.432	13.277	s.
C. 20 hour observation				
Overall: 8, 11, 14, 17, 21, 23C	5	108.838	15.086	s.
Group 1: 8, 11, 14, 17, 21C	4	25.73	13.277	s.
Difference: 23C - 8 + 11 + 14 + 17 + 21C	5	83.21	15.086	s.
Group 2: 11, 14, 17, 21C	3	9.454	11.345	n.s.
Difference: 8 + 23C - 11 + 14 + 17 + 21C	5	78.532	15.086	s.
D. 28 hour observation				
Overall: 8, 11, 14, 17, 21, 23C	5	78.012	15.086	s.
Group 1: 21, 23C	1	4.444	6.635	n.s.
Group 2: 8, 11, 14, 17C	3	18.6027	11.345	s.
Difference: Group 1 vs. Group 2	5	54.965	15.086	n.s.
E. 40 hour observation				
Overall: 8, 11, 14, 17, 21, 23C	5	218.219	15.086	s.

*s. chi-square value is significant (i.e., $x^2 > x^2_{\infty}$)

**n.s. chi-square value is not significant (i.e., $x^2 < x^2_{\infty}$)

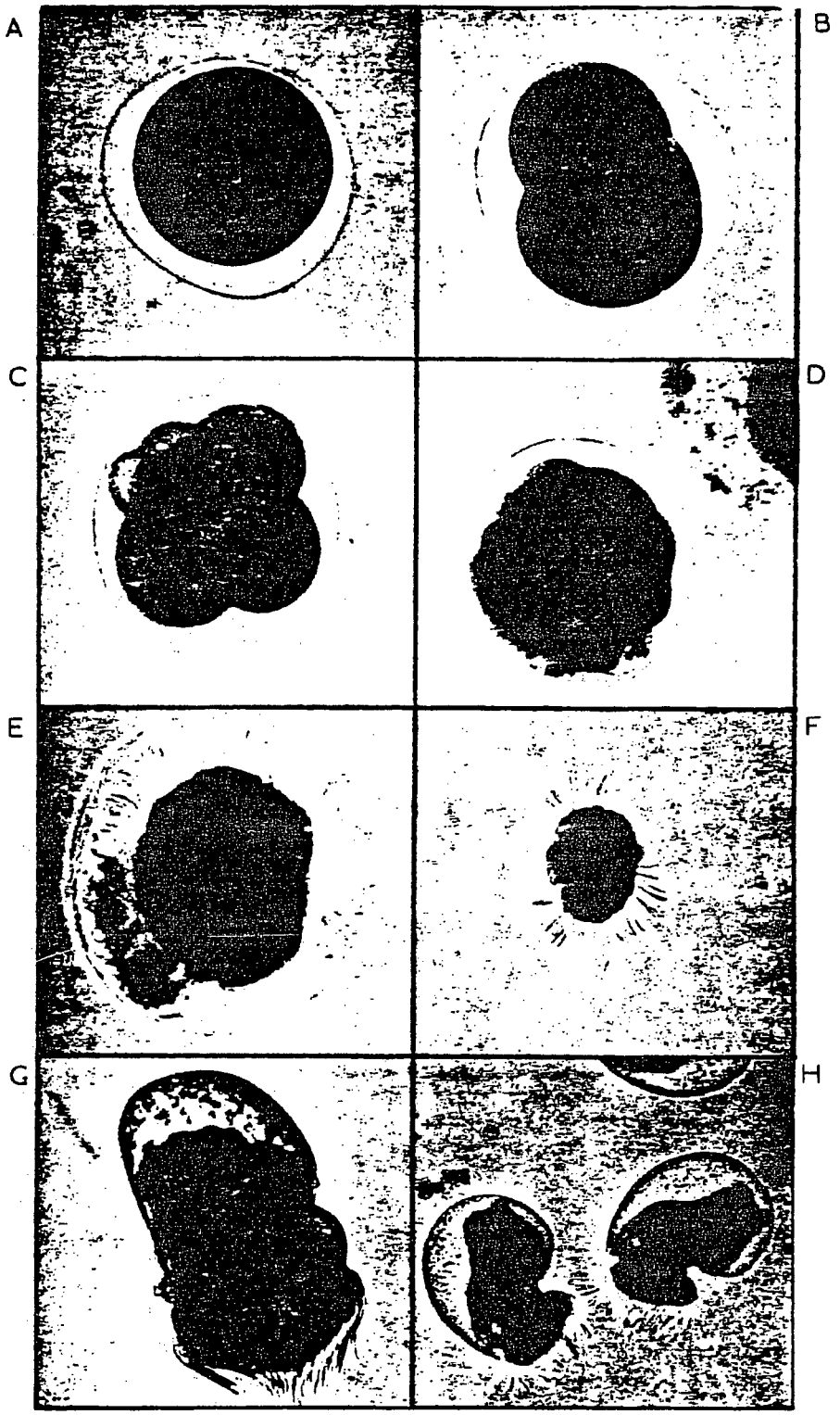
*** degrees of freedom used is equal to total n - 1 samples to control for erroneous inferences when making comparisons (see Fleiss 1973).

treatments, 21 and 23 C. Levels of mortality in the 40-hour observation, although highly significant, were not tested further, since a posteriori groupings of the treatments in the 28-hour observation were believed to reflect the optimal and maximum temperature ranges for black abalone. This is substantiated by the steep rise in mortality from 17 to 21 C as shown in FIGURE 3.5-1. An intermediate temperature treatment would have aided in determination of the upper limit of the optimum range.

Development

Larval stages were those identified in the literature by work on related species of Haliotis (Crofts 1937, Ino 1952, Leighton 1974 and Morse et al. 1979b). The photographs in FIGURE 3.5-2 represent examples of some of the normal developmental stages observed in black abalone in this series of experiments. Not all developmental stages are shown, but as is evident by comparison with the literature, development in black abalone follows the normal molluscan pattern reported in other haliotids. The most notable and conspicuous feature of black abalone larvae is the dark green yolk evident until the veliger is fully formed.

A summary of the observations on larval development is presented in TABLE 3.5-7. The modal stage for each of the samples was plotted against temperature for each of the observation times in the prehatch cohort treatments (FIGURE 3.5-3). Normal development occurred from 10 to 20 C for larvae in the prehatch and early posthatch stages. As is exhibited by the series of curves in FIGURE 3.5-3, developmental rate is highly temperature dependent. Larval development proceeded at an increasing rate with increasing temperature until total mortality occurred (see previous subsection). Rate of development dropped rapidly from about 14 to 8 C. A majority of the larvae in the 10 C treatment had still not hatched by the 40-hour observation, whereas in the 14.5 and 17 C treatments hatching occurred at approximately 24 hours. These hatching times agree fairly closely with those obtained by Leighton (1974) and Morse et al. (1979b) on H. rufescens.



A = unfertilized egg B = 2-cell embryo C = 8-cell embryo
D = morula E = pre-hatch trochophore F = swimming trochophore
G = veliger with early "cap" shell H = pre-operculate veligers.
Magnification varies.

FIGURE 3.5-2

PHOTOGRAPHS OF NORMALLY DEVELOPED LARVAE AT EIGHT STAGES:
BLACK ABALONE LARVAL DEVELOPMENT

TABLE 3.5-7

SUMMARY OF DEVELOPMENT DATA:
BLACK ABALONE LARVAL DEVELOPMENT

Temperature (C)	Sample (hours)	Stage: 2-cell Embryo	4-cell Embryo	8-cell Embryo	16-cell Embryo	Morula	Blastula	Early Gastrula	Rotating Gastrula	Prehatch Trochophore	Swimming Trochophore	Capshell Veliger	Inflate shell Veliger	Early operculate Veliger	Incipient cephalic tentacle	Mid-formed cephalic tentacle
PREHATCH COHORT																
8	3	9	31	3												
	10	—	—	—												
	20					41										
	27					8	16	2								
	40						8	6	1							
11	3	2	14	23	3											
	10				3	38	2									
	20						6	20	29							
	27						11	28	25	8						
	40									99	16	2				
14.5	3	3	9	23	5	1										
	10	1	2				10	30	1							
	20								9	31						
	27	1						1		45	17					
	40											1	104			
17	3	3	5	27	9	2										
	10							12	36							
	20					1			11	22	4					
	27									3	10	11	11			
	40											1	25			
21	3	4	8	16	14	5										
	10	3						1	18	20						
	20										9	10	5			
	27										2		13			
	40	—	—	—	—	—	—	—	—	—	—	—	—			
23.3	3	1	3	6	23	3	3									
	10	2					2	3								
	20						3	1			5					
	27							1								
	40															
POSTHATCH COHORT																
9	80												4	48		
	100														50	
	116															30
13	80												3	40		
	100															25
	116															2
15.5	80														42	
	100															1
	116															
18	80														62	
	100															4
	116															2
20	80														37	
	100															13
	116															
23	80													4	49	
	100															5
	116															1

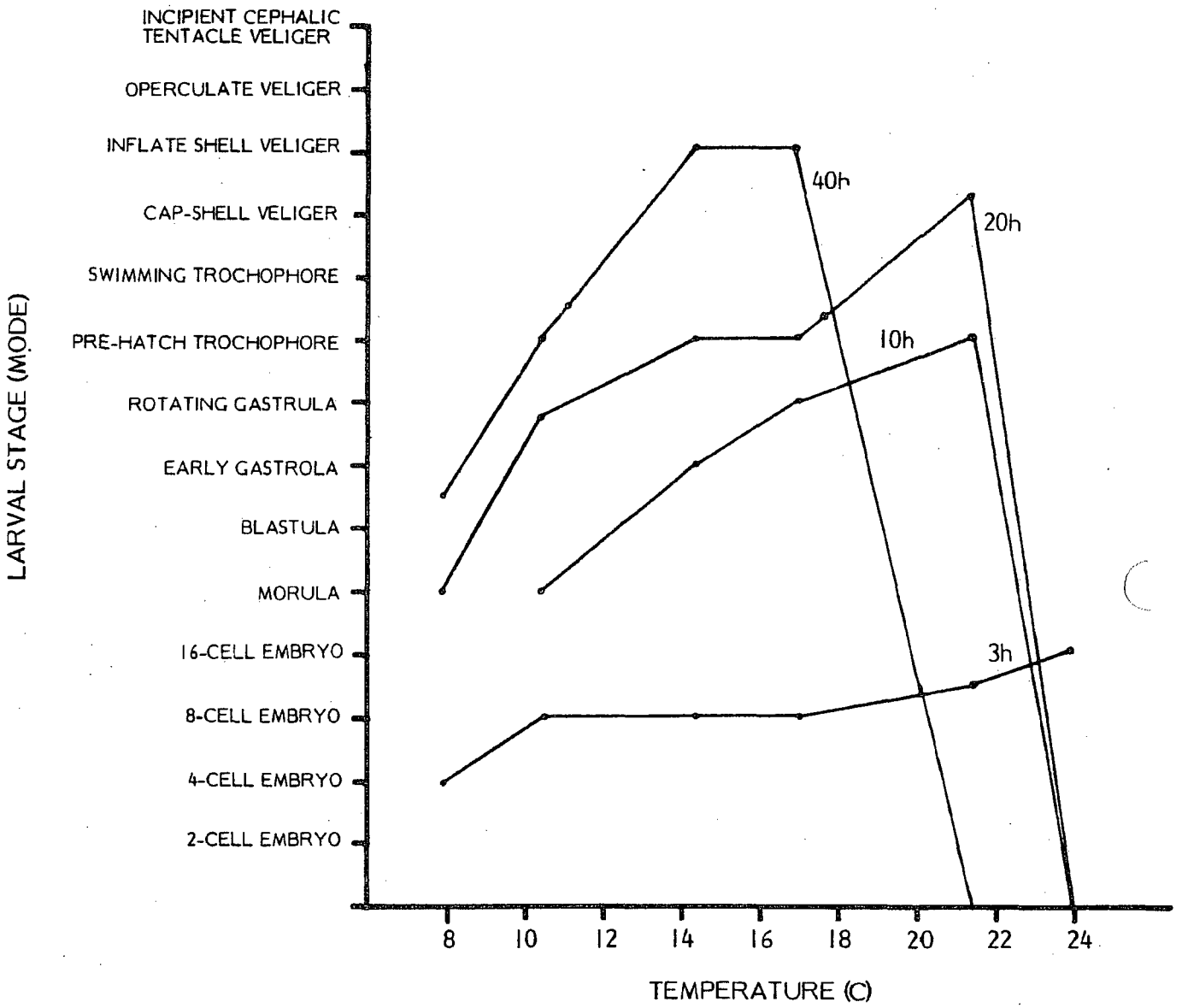


FIGURE 3.5-3

LARVAL STAGE VERSUS TEMPERATURE
AT 3, 10, 20 AND 40 HOURS:
BLACK ABALONE LARVAL DEVELOPMENT

Data for the posthatch cohort are also included in TABLE 3.5-7. This cohort was added to the gradient following hatching in the ambient control. Upon inspection it can be seen that mortality does occur at the lower temperature extremes in the posthatch cohort as it did in the prehatch cohort. In other experiments performed by us, it has been shown that larvae introduced to extremes of temperature at a later post-hatching stage of development showed increased temperature tolerance. It is possible that this also occurred in the posthatch cohort. In this experiment high mortality levels resulting from predation by ciliated protozoans and bacteria unfortunately precluded further analysis of the data relative to this point.

3.5.1.3 DISCUSSION

The optimum temperature for early development of black abalone from fertilized egg through mid-formed cephalic tentacle larvae is within the range 10 to 22 C (50 to 72 F) based on rates of mortality and development. Rate of development itself actually reaches a peak at the higher temperatures but increasing rates of mortality at these temperatures with time results in total loss of the experimental population. There is some indication that larvae of later developmental stages subsequently subjected to comparable temperatures are somewhat more heat tolerant than are the early embryonic stages. The data indicate that exposures of less than three hours in the 8 to 24 C (46 to 75 F) range do not result in immediately apparent mortality but that after 10 hours continuous exposure the temperature range with less than 50 percent mortality shrinks to about 10 to 22 C (50 to 72 F) and by 40 hours to 10 to 16 C (50 to 61 F).

Developmental rates observed in black abalone in these experiments are comparable to those reported for red abalone (H. rufescens) by Leighton (1974). The optimal and maximum temperature ranges observed are also compatible with results obtained by us in experiments on red abalone. The apparently higher maximum temperature of 23 C (73 F) reported by Leighton (1974) could be attributed to latitudinal variations in temperature tolerance within a species.

3.5.2 GROWTH

An experiment was conducted to investigate the effects of water temperature and diet on the growth of black abalone. The purpose of the experiment was to measure under laboratory conditions the growth rate of abalone cultured in different combinations of temperature and available algal diets and to determine the levels of these two factors which cause significant changes in the growth response. The information gained in this experiment will enable an evaluation of the potential effect of changing field conditions which might be induced by the discharge of waste heat to Diablo Cove. This experiment was performed in concert with growth experiments of three other molluscan species using the same methods and culture facilities (red abalone, see SECTION 3.6.3; Tegula brunnea, see SECTION 3.10.2; and Tegula funebralis, see SECTION 3.10.3).

The growth of black abalone under natural conditions has been investigated by Wright (1975) and Leighton and Boolootian (1963). Growth rate estimates in these studies were based on measured change in shell length of tagged animals recovered after approximately 12 months at large. In addition to the growth studies of tagged animals, Leighton and Boolootian (1963), cultured field collected specimens in a southern California marine laboratory in order to investigate the abalone's feeding characteristics based on algal preference and growth response to a wide variety of algal diets. They found that black abalone preferred Egregia laevigata, Gigartina canaliculata and Pelvetia fastigiata ranked as first, second and third choices based on algal preference values. However, the top three weight promoting algal species were Macrocystis pyrifera, Gigartina canaliculata and Pelvetia fastigiata. Macrocystis ranked sixth in the algal preference experiments.

Black abalone stomach contents of field collected animals from Palos Verdes and Pt. Dume examined by Leighton and Boolootian (1963) showed a preponderance of brown algal fragments with lesser amounts of Gigartina canaliculata, Pelvetia fastigiata and trace amounts of marcoalgae, diatoms, bryozoams, foraminifera, and sponges. Fecal pellets contained fragments of coralline algae, Phyllospadix and undigested diatoms.

Growth rates of black abalone reported by Leighton and Boolootian (1963) were contrasted by Wright (1975) to her results of tagged abalone growth rates from a population studied on Santa Cruz Island offshore of Santa Barbara. Wright found average annual increases at 14.78 mm and 14.64 mm for animals in the 30 to 50 mm and 51 to 80 mm size classes, respectively. In contrast, Leighton and Boolootian reported a 20 mm average annual rate of increase for animals in the 50-100 mm size class. Both investigators reported significant reductions in growth rates at the 80 mm and above size classes as well as an overall unpredictability in individual abalone cyclical growth rates. Growth rates reported by Wright (1975) and Leighton and Boolootian (1963) are analyzed and compared in a discussion of this experiment's results.

The thermal tolerances of adult black abalone (100-150 mm) were investigated in a series of 96-hour median effective temperature tests on sample populations collected in intertidal areas immediately north and south of Diablo Cove. Ecological death, the loss of the ability to adhere to a surface, response to elevated temperature occurred abruptly with 96-hour ET50 values of 26.6 and 27.2 C in the respective 11 and 16 C acclimated animals with tidal exposure cycles. Black abalone without tidal exposure exhibited 96-hour ET50 values of 26.1 and 27.4 C acclimated at 11 and 16 C, respectively. These findings were employed to select the sublethal temperature range in the present growth studies.

The following description of the methods and materials employed in the black abalone growth experiments summarize the key points of the experimental design. For additional details on laboratory methods the reader is referred to the laboratory procedure control document included in this report as APPENDIX B (Procedure 310) and protocol, see APPENDIX C (experiment 10a).

3.5.2.1 METHODS

The experimental design of the black abalone growth studies included 90 day observations of shell length increases among four species of snails: *Haliotis rufescens*, *H. cracherodii*, *Tegula brunnea* and *T. funebralis*. In addition,

changes in animal body component weights (foot, visceral mass and shell) and cross-sectional dimension of gonads and digestive glands were also measured. Each species' growth response was tested at six temperatures (23.8, 21.0, 17.8, 14.9, 12.1 and 12.0 C) and with three diets (brown, red and red/brown algae).

Approximately 600 test specimens of black abalone were collected from the intertidal zone just south of Diablo Cove from 13-30 November 1978. The abalone were carefully examined for any injury from collection or handling and held for observation for a period of 30+ days in running ambient seawater. During this time they were fed an excess amount of Macrocystis. At the end of the initial observation period the animals were individually tagged, weighted and placed in fine mesh 2 x 3 x 1 ft plastic screen holding pans located in the appropriate running seawater 4 x 8 ft test tanks. Initial shell length and body weight were recorded for each tagged individual. Test tank temperatures were slowly increased at the rate of 1 C/day until the appropriate test temperature was achieved. A total of 18 treatment groups (6 temperatures x 3 diets) each containing 30 individuals provided a total experimental population of approximately 540 abalone. An effort was made to place similar size distributions of abalone in each treatment cage; size range in each cage was approximately 10-15 mm. Test temperatures in each 4 x 8 ft tank were electronically controlled to within ± 0.1 C of the specified test temperature throughout the course of the 90 day observation period. An unplanned power outage at 0719 on 10 January (day 6) caused larger variations as follows: the 12.0 C tank ranged up to 13.2 C for a 2-hour period, the 14.9 C tank ranged from 13.3 to 14.6 C for a 16-hour period, the 17.8 C tank ranged from 13.3 to 15.7 C for a 34-hour period, the 21.0 C tank ranged from 15.1 to 18.7 C for 25 hours, and the 23.8 C tank ranged from 14.2 to 20.7 C for 50 hours. The 90-day mean temperature for each treatment will be used for identification in this report. The means, standard deviations and ranges were: 12.1 ± 0.7 (9.9-13.7) (ambient), 12.0 ± 0.3 (11.7-13.6), 14.8 ± 1.3 (13.3-19.8), 17.9 ± 0.4 (13.3-18.4), 21.0 ± 1.2 (15.1-21.8) and 23.8 ± 0.9 (14.2-24.2). Flow rates in the test tanks were maintained at an approximately constant rate of 22.7 liters/min (6 gpm).

The three algal test diets were composed of brown algae (Nereocystis luetkeana and Pterygophora californica), red algae (Iridaea flaccida and Botryoglossum farlowianum) and a mixed algal diet composed of roughly equal amounts of the four preceding red and brown species. In addition, diatoms growing on the walls of the test containers were also available as food to all the treatment groups. The algal species of the diets were selected to represent the species of algae which commonly compose the algal drift found along the Diablo Cove shoreline. It was suspected that based on previous studies (Leighton and Boolootian 1963, Paul et al. 1977) Nereocystis and Pterygophora would be the preferred algal diet and that in the mixed red and brown diet that the abalone would consume only brown algae if fed in excess with a red and brown mixture. Therefore the ration of brown algae in the mixed red and brown diet was presented at one-half the consumption rate of the abalone given only brown algae. This value was monitored at the outset of the experiment and on day 29, was fixed as follows: brown algal diet, 300 g Nereocystis and 100 g Pterygophora; red algae diet, 100 g each of Iridaea and Botryoglossum; and mixed diet, 150 g Nereocystis and 50 g each of Pterygophora, Iridaea and Botryoglossum. The red and brown algal diets were fed to excess in each treatment group.

Measurements of shell growth and body weight were conducted at the beginning and at 45-day and 90-day intervals. In addition, subsamples of body component weights in each test population were collected at the beginning and end of the experiment. At the completion of the 90-day tests the abalone were returned to the intertidal region in the Diablo Cove area or utilized in subsequent temperature preference experiments then returned to the shoreline.

3.5.2.2 RESULTS

The results of the 90-day growth experiments indicated detectable differences in shell length increases among all treatment groups of temperature and diet combination. Smaller changes in body weight and body component weights were observed with only slight differences among treatment groups. Therefore, the following analysis of results examine only shell length increases for the purpose of this report.

The amount of shell size increase of an individual abalone is determined by a number of environmental factors and is partially predetermined by the size of the individual. In order to normalize the growth data, the analyses were performed on the calculated variable, $\Delta LN = LN_3 - LN_1$; where LN_1 = length of abalone at the initial measurement and LN_3 = length of abalone at the end of the 90-day growth period. Secondly, in order to make any statistical inference regarding the experimental outcome among the treatment groups, it must be assumed that all treatment groups contained abalone with shell lengths of equal variance. As described in the preceding methods, an attempt was made at the start of the experiment to sort the size classes among treatments in order to achieve equal treatment group variance in initial shell length. A test of the homogeneity of variance among the initial treatment group sizes based on an F-ratio showed that there were no significant differences among the treatment groups at the $p = 0.98$. Similar tests using Cochran's C statistic (0.0738, $p = 1.00$), Bartlett-Box F statistic (0.575, $p = 0.913$) and MAX^2/MIN^2 (1.960) also showed no significant differences between the treatment group's initial size variations. Therefore a test of the variance in length increase at the end of the 90-day growth period will identify any significant difference among the test populations due to the experimental factors, diet and temperature. Because the experiment was a fully nested design, an ANOVA of the two treatment levels enables an inference as to which factor is most important to black abalone growth.

A two way ANOVA was performed on the variable ΔLN for all treatment groups (6 temperatures x 3 diets). A total of 539 abalone ΔLNs were analzyed; 87 (16.1 percent) of the cases were missing due to mortality losses during the experiment. A significant amount of variation in shell length increases ($p = .999$) was explained by the main factors temperature and diet, and their two way interaction. Differences in length increase between the three diets were significant at just less than the 90 percent probability level ($p = 0.897$). The effect of temperature on shell length increase was much greater than the effect of diet; differences among test temperatures were significant at greater than 99.9 percent probability level. The complete results of the ANOVA are presented in TABLE 3.5-8.

TABLE 3.5-8

ANOVA OF GROWTH (IN MM) AFTER 90 DAYS AT SIX TEMPERATURES AND THREE DIETS: BLACK ABALONE GROWTH

```
* * * * * * * * * * * A N A L Y S I S   O F   V A R I A N C E * * * * * * * * * *
                LNIN
             BY DIET
                TEMP
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
                                        SUM OF                    MEAN          SIGNIF
SOURCE OF VARIATION                     SQUARES      DF          SQUARE     F    OF F

MAIN EFFECTS                            305.398       6          50.900   7.506   .000
   DIET                                 30.829       2          15.415   2.285   .103
   TEMP                                 281.794      4          70.449  10.444   .000

2-WAY INTERACTIONS                      86.633       8          10.829   1.605   .121
   DIET      TEMP                      86.633       8          10.829   1.605   .121

EXPLAINED                               392.031     14          28.002   4.151   .000

RESIDUAL                               2947.692    437           6.745

TOTAL                                  3339.723    451           7.405
     539 CASES WERE PROCESSED.
      87 CASES ( 16.1 PCT) WERE MISSING.
```

GROUP	COUNT	MEAN	STANDARD DEVIATION	STANDARD ERROR	MINIMUM	MAXIMUM	95 PCT CONF INT FOR MEAN		
GRP01	27	2.6333	1.8605	.3619	-.6000	7.8000	1.8894	TO	3.3772
GRP02	23	1.9783	1.5282	.3187	-.2000	6.1000	1.3174	TO	2.6391
GRP03	24	3.3069	2.5369	.4711	-.5000	9.6000	2.3419	TO	4.2719
GRP04	27	3.0926	2.1064	.4054	-2.0000	7.9000	2.2593	TO	3.9259
GRP05	26	3.7231	2.4087	.4724	-.4000	8.7000	2.7502	TO	4.6960
GRP06	21	2.8810	2.0853	.4551	-.8000	6.8000	1.9317	TO	3.8302
GRP07	24	2.0125	1.2529	.2556	-1.4000	4.2000	1.4834	TO	2.5416
GRP08	25	1.6880	1.3602	.2720	-.7000	4.9000	1.1265	TO	2.2495
GRP09	24	2.0625	1.6860	.3441	-.6000	4.9000	1.3506	TO	2.7744
GRP10	29	4.8931	2.3531	.4370	.3000	8.9000	3.9980	TO	5.7882
GRP11	23	2.7304	2.7036	.5636	-.2000	11.5000	1.5612	TO	3.8996
GRP12	25	2.1680	1.7418	.3484	-.1000	5.2000	1.4490	TO	2.8870
GRP13	27	2.2704	1.5735	.3028	-.4000	6.0000	1.6479	TO	2.8928
GRP14	29	2.5793	1.9549	.3630	-.5000	6.7000	1.8357	TO	3.3229
GRP15	23	3.1304	2.1482	.4479	-.4000	7.1000	2.2015	TO	4.0594
GRP16	25	4.6640	2.8582	.5716	.3000	10.5000	3.4842	TO	5.8438
GRP17	23	4.2391	2.5584	.5335	.6000	9.2000	3.1328	TO	5.3454
GRP18	22	3.2818	2.2302	.4755	.3000	7.7000	2.2930	TO	4.2706
TOTAL	452	2.9792	2.2601	.1063	-2.0000	11.5000	2.7703	TO	3.1881
	FIXED EFFECTS MODEL		2.1049	.0990			2.7846	TO	3.1738
	RANDOM EFFECTS MODEL		.9483	.2235			2.5076	TO	3.4508

RANDOM EFFECTS MODEL - ESTIMATE OF BETWEEN COMPONENT VARIANCE .7163

TESTS FOR HOMOGENEITY OF VARIANCES

```
COCHRANS C = MAX. VARIANCE/SUM(VARIANCES) =   .1020, P =  .041 (APPROX.)
BARTLETT-BOX F =                              2.472, P =  .001
MAXIMUM VARIANCE / MINIMUM VARIANCE =         5.204
```

*TREATMENT GROUP CODES	12.0	12.1	14.8	17.9	21.0	23.8	TEMP C
BROWN ALGAL DIET	1	2	3	4	5	6	
RED ALGAL DIET	7	8	9	10	11	12	
MIXED ALGAL DIET	13	14	15	16	17	18	

Three multiple range test procedures, LSD (least significant difference) ($p = 0.010$), SNK (Student, Newman, Keuls), and Scheffe (0.01) were selected to analyze the set of treatment groups mean ΔLNs shown in TABLE 3.5-8. Each procedure is slightly less sensitive and more robust from the LSD to the Scheffe statistic.

The results of the LSD test separated 5 homogeneous treatment group subsets (subsets of groups whose highest and lowest means do not differ by more than the shortest significant range for a subset of that size, range = 3.66). The treatment groups contained in the five subsets are listed in TABLE 3.5-9. A wide range of overlap among groups occurred using this sensitive test. In general the groups were composed of temperature sets of the extreme temperatures (12 and 23 C) and the mid-range temperatures (14 to 21 C). Within these groups two additional sets were partially defined by diet groupings of the mixed and brown algal diets (1 and 3) and the red algal diet (2). The results of the SNK test (less sensitive, more robust than LSD) defined two subsets as also shown in TABLE 3.5-10. The SNK procedure subsets also separated on the basis of treatment group temperatures; subset 1 contained the lower and upper temperature treatment groups and subset 2 contained the mid-range temperature treatment groups. Scheffe's procedure defined a single subset and the results are not tabulated.

The mean values of shell length increase were plotted as shown in FIGURE 3.5-4. The pattern of results suggests optimum growth between test temperatures of 14.8 and 21.0 C and an optimum growth temperature very close to 17.9 C.

Results for the brown algal diet treatment group at 17.9 C produced an average length increase much below the other mean increases for the red and mixed algal diets at 17.9 C. A comparison of size and sex distribution among the three treatment groups identified considerable differences. The brown algal diet group contained significantly fewer female and immature abalone. A regression analysis of the growth rate of these three sex categories at 17.9 C temperatures indicated some differences in their respective growth rates as shown in FIGURE 3.5-5. In addition the three sex classes are also linked to size

TABLE 3.5-9

LSD TEST OF GROWTH DATA (IN MM) AT 90 DAYS BY TREATMENT GROUP: BLACK ABALONE GROWTH

```
LSD PROCEDURE
RANGES FOR THE  .010 LEVEL -

          3.66  3.66  3.66  3.66  3.66  3.66  3.66  3.66  3.66  3.66  3.66  3.66  3.66  3.66  3.66

          3.66  3.66

THE RANGES ABOVE ARE TABLE RANGES.  THE VALUE ACTUALLY COMPARED WITH MEAN(J)-MEAN(I) IS..
        1.8884 * RANGE * SQRT(1/N(I) + 1/N(J))
  HOMOGENEOUS SUBSETS   (SUBSETS OF GROUPS, WHOSE HIGHEST AND LOWEST MEANS DO NOT DIFFER BY MORE THAN THE SHORTEST
                         SIGNIFICANT RANGE FOR A SUBSET OF THAT SIZE)

SUBSET  1

GROUP     GRP08     GRP02     GRP07     GRP09     GRP12     GRP13     GRP14     GRP01
MEAN      1.6880    1.9783    2.0125    2.0625    2.1680    2.2704    2.5793    2.6333

GROUP     GRP11     GRP06     GRP04     GRP15
MEAN      2.7304    2.8810    3.0926    3.1304
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

SUBSET  2

GROUP     GRP02     GRP07     GRP09     GRP12     GRP13     GRP14     GRP01     GRP11
MEAN      1.9783    2.0125    2.0625    2.1680    2.2704    2.5793    2.6333    2.7304

GROUP     GRP06     GRP04     GRP15     GRP18     GRP03
MEAN      2.8810    3.0926    3.1304    3.2818    3.3069
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

SUBSET  3

GROUP     GRP13     GRP14     GRP01     GRP11     GRP06     GRP04     GRP15     GRP18
MEAN      2.2704    2.5793    2.6333    2.7304    2.8810    3.0926    3.1304    3.2818

GROUP     GRP03     GRP05
MEAN      3.3069    3.7231
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

SUBSET  4

GROUP     GRP11     GRP06     GRP04     GRP15     GRP18     GRP03     GRP05     GRP17
MEAN      2.7304    2.8810    3.0926    3.1304    3.2818    3.3069    3.7231    4.2591
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

SUBSET  5

GROUP     GRP15     GRP18     GRP03     GRP05     GRP17     GRP16
MEAN      3.1304    3.2818    3.3069    3.7231    4.2591    4.6680
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

SUBSET  6

GROUP     GRP05     GRP17     GRP16     GRP10
MEAN      3.7231    4.2591    4.6680    4.8931
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
```

*TREATMENT GROUP CODES	12.0	12.1	14.8	17.9	21.0	23.8	TEMP C
BROWN ALGAL DIET	1	2	3	4	5	6	
RED ALGAL DIET	7	8	9	10	11	12	
MIXED ALGAL DIET	13	14	15	16	17	18	

TABLE 3.5-10

SNK TEST OF GROWTH DATA (IN MM) AT 90 DAYS BY TREATMENT GROUP: BLACK ABALONE GROWTH

STUDENT-NEWMAN-KEULS PROCEDURE
RANGES FOR THE .050 LEVEL -

2.81 3.34 3.65 3.88 4.05 4.19 4.31 4.41 4.49 4.57 4.64 4.71 4.76 4.82 4.87

4.91 4.96

THE RANGES ABOVE ARE TABLE RANGES. THE VALUE ACTUALLY COMPARED WITH MEAN(J)-MEAN(I) IS..
1.4884 * RANGE * SQRT(1/N(I) + 1/N(J))
HOMOGENEOUS SUBSETS (SUBSETS OF GROUPS, WHOSE HIGHEST AND LOWEST MEANS DO NOT DIFFER BY MORE THAN THE SHORTEST SIGNIFICANT RANGE FOR A SUBSET OF THAT SIZE)

SUBSET 1

GROUP	GRP08	GRP02	GRP07	GRP09	GRP12	GRP13	GRP14	GRP01
MEAN	1.6880	1.9783	2.0125	2.0625	2.1680	2.2704	2.5793	2.6333
GROUP	GRP11	GRP06	GRP04	GRP15	GRP18	GRP03		
MEAN	2.7304	2.8610	3.0926	3.1304	3.2818	3.3069		

SUBSET 2

GROUP	GRP02	GRP07	GRP09	GRP12	GRP13	GRP14	GRP01	GRP11
MEAN	1.9783	2.0125	2.0625	2.1680	2.2704	2.5793	2.6333	2.7304
GROUP	GRP06	GRP04	GRP15	GRP18	GRP03	GRP05		
MEAN	2.8610	3.0926	3.1304	3.2818	3.3069	3.7231		

SUBSET 3

GROUP	GRP14	GRP01	GRP11	GRP06	GRP04	GRP15	GRP18	GRP03
MEAN	2.5793	2.6333	2.7304	2.8610	3.0926	3.1304	3.2818	3.3069
GROUP	GRP05	GRP17						
MEAN	3.7231	4.2391						

SUBSET 4

GROUP	GRP06	GRP04	GRP15	GRP18	GRP03	GRP05	GRP17	GRP16
MEAN	2.8610	3.0926	3.1304	3.2818	3.3069	3.7231	4.2391	4.6040

SUBSET 5

GROUP	GRP18	GRP03	GRP05	GRP17	GRP16	GRP10
MEAN	3.2818	3.3069	3.7231	4.2391	4.6040	4.8931

*TREATMENT GROUP CODES	12.0	12.1	14.8	17.9	21.0	23.8	TEMP C
BROWN ALGAL DIET	1	2	3	4	5	6	
RED ALGAL DIET	7	8	9	10	11	12	
MIXED ALGAL DIET	13	14	15	16	17	18	

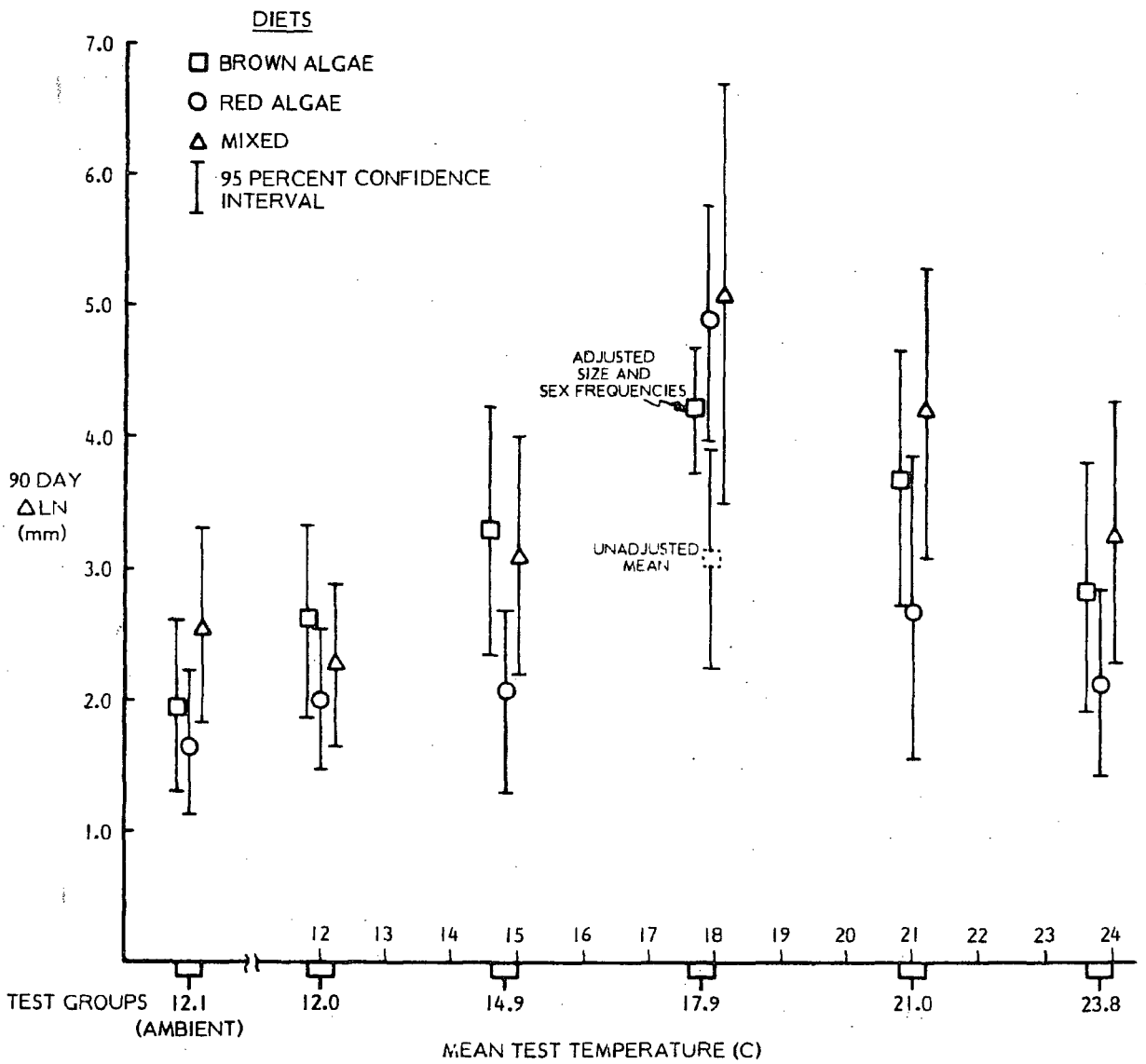


FIGURE 3.5-4

90 DAY GROWTH VERSUS TEMPERATURE
AT THREE DIETS: BLACK ABALONE GROWTH

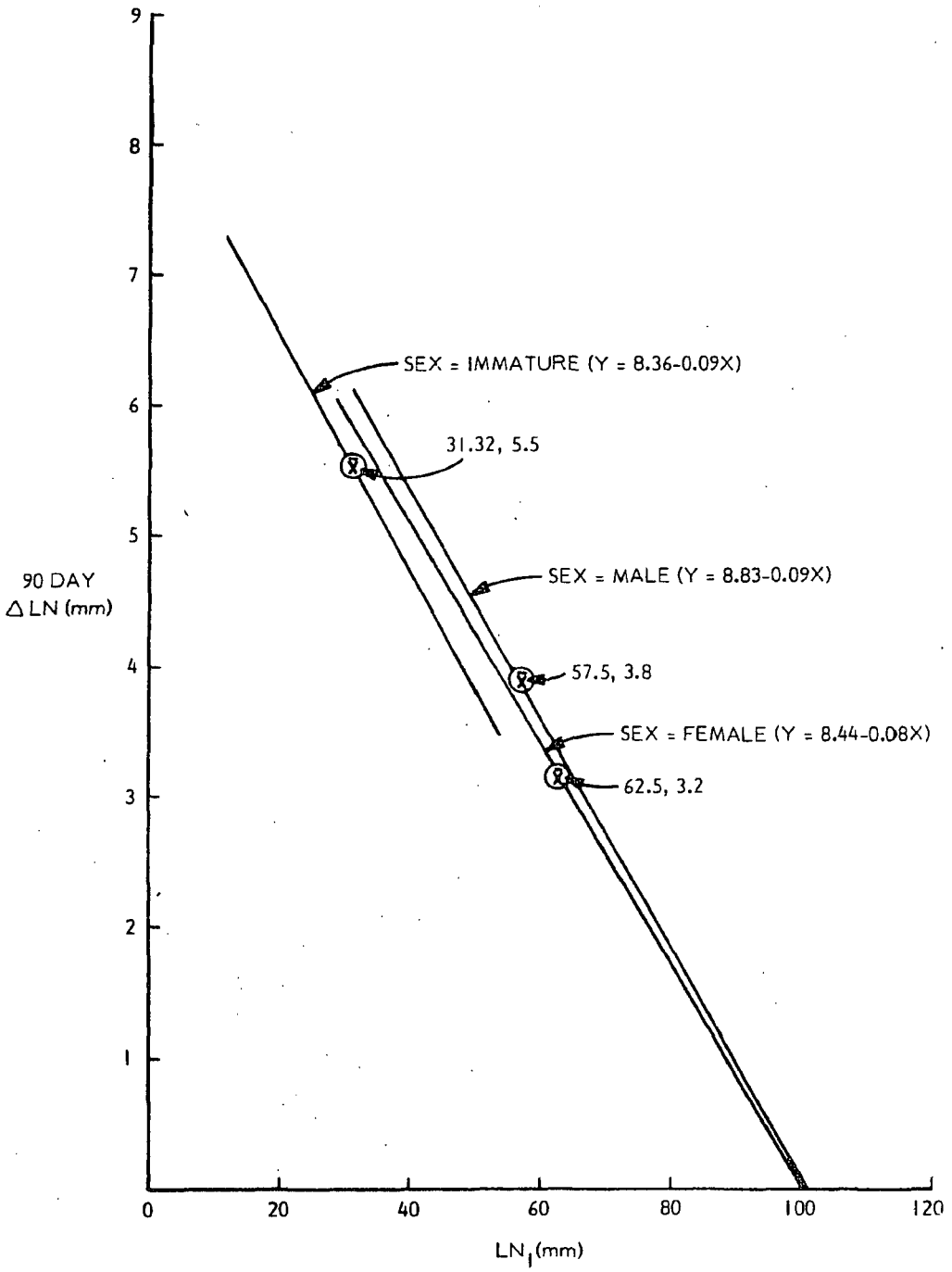


FIGURE 3.5-5

CHANGE IN LENGTH (Δ LN) VERSUS INITIAL LENGTH (LN_I) AFTER 90 DAYS AT 17.9 C BY SEX: BLACK ABALONE GROWTH

Data for all diets at 17.9 C were combined.

frequencies. A regression analysis of the length of increases vs. the initial length of each test animal showed a significant relationship between the variables as illustrated in FIGURE 3.5-5. Using the relationships of size and sex to growth rates from the 17.9 C treatment group, the mean length increase for the brown algal diet animals at 17.9 C was adjusted by the substitution of average growth increase values for abalone of appropriate sizes and sex to achieve similar size and sex distribution among the diet groups. The resulting adjusted mean length increase for the brown algal diet is also shown in FIGURE 3.5-4 and more nearly matches the other diet treatment group increases at 17.9 C.

The effect of diet on growth appears to be smaller than temperature effects and more variable (as shown previously in the ANOVA). The growth trends shown in FIGURE 3.5-4 also indicate reduced growth on a red algal diet at upper temperatures. It can be postulated from this trend that the red algae were nutritionally inadequate to sustain increased metabolism due to elevated temperatures and promote growth at the new metabolic potentials.

The 90-day increase in length for each test animal was regressed against the animal's initial length. The analyses were performed for each treatment group and included an average of 27 animals. The resulting equations are illustrated by the brown, red and mixed algal diets in FIGURES 3.5-6 to 3.5-8, respectively. Variance about these regression lines is relatively wide and no statistical comparisons of the B_1 values were performed. By inspection, the major differences among the growth curves include slower growth at 12.0 and 12.1 C and in the red algal diet treatments. As discussed previously the highest growth rates were found in the 17.9 C temperature groups fed the brown and mixed red/brown diets. Much of the growth variation within the treatment groups may be due to differences in the initial size (and possibly sex) distributions within each group. However, it was also found that some number of small abalone for no apparent reason exhibited practically no growth during the experiment. These animals may have been damaged or behaviorally disturbed by the collection process.

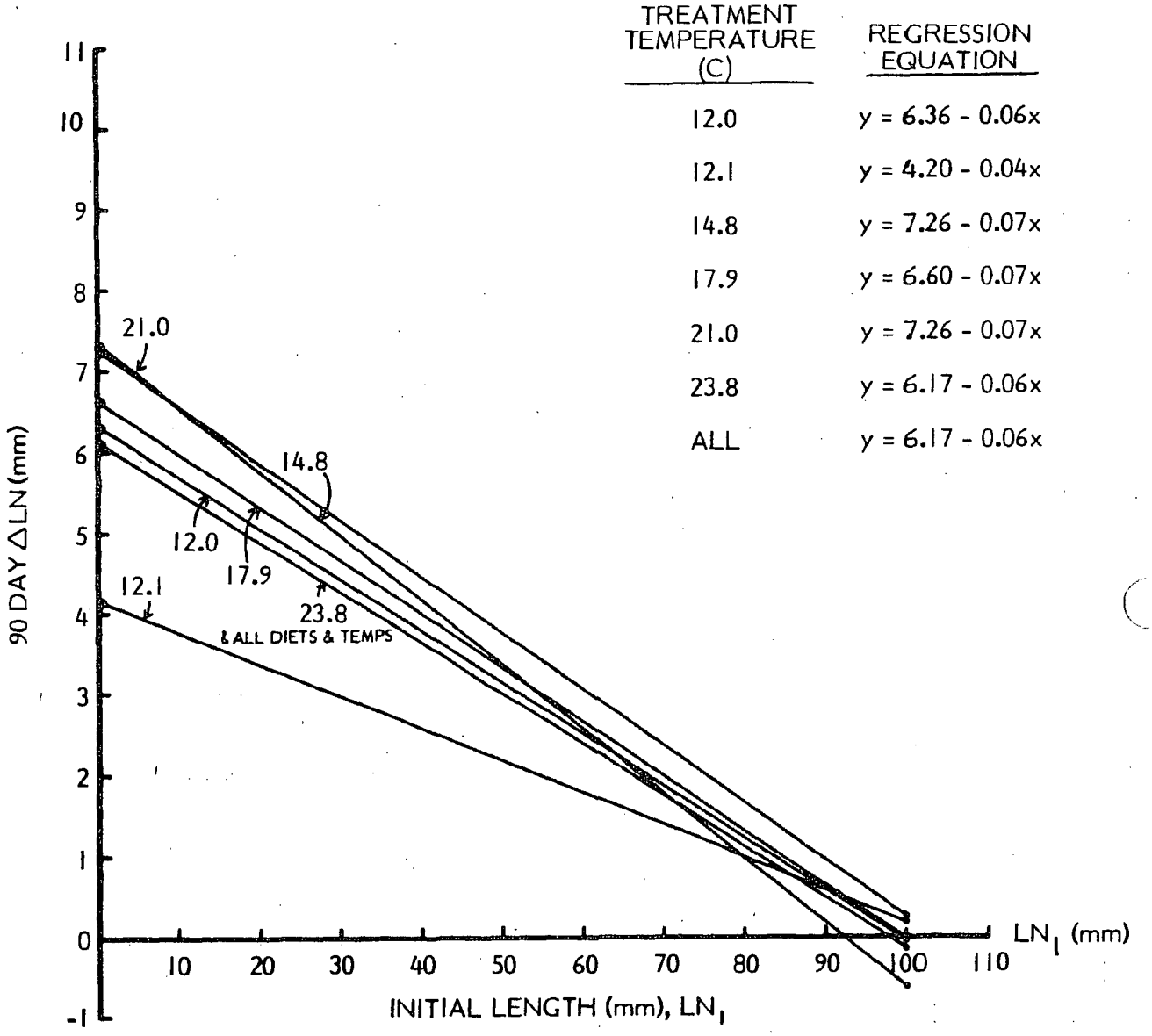


FIGURE 3.5-6

90 DAY CHANGE IN LENGTH VERSUS INITIAL LENGTH AT SIX TEMPERATURES WITH BROWN ALGAL DIET: BLACK ABALONE GROWTH

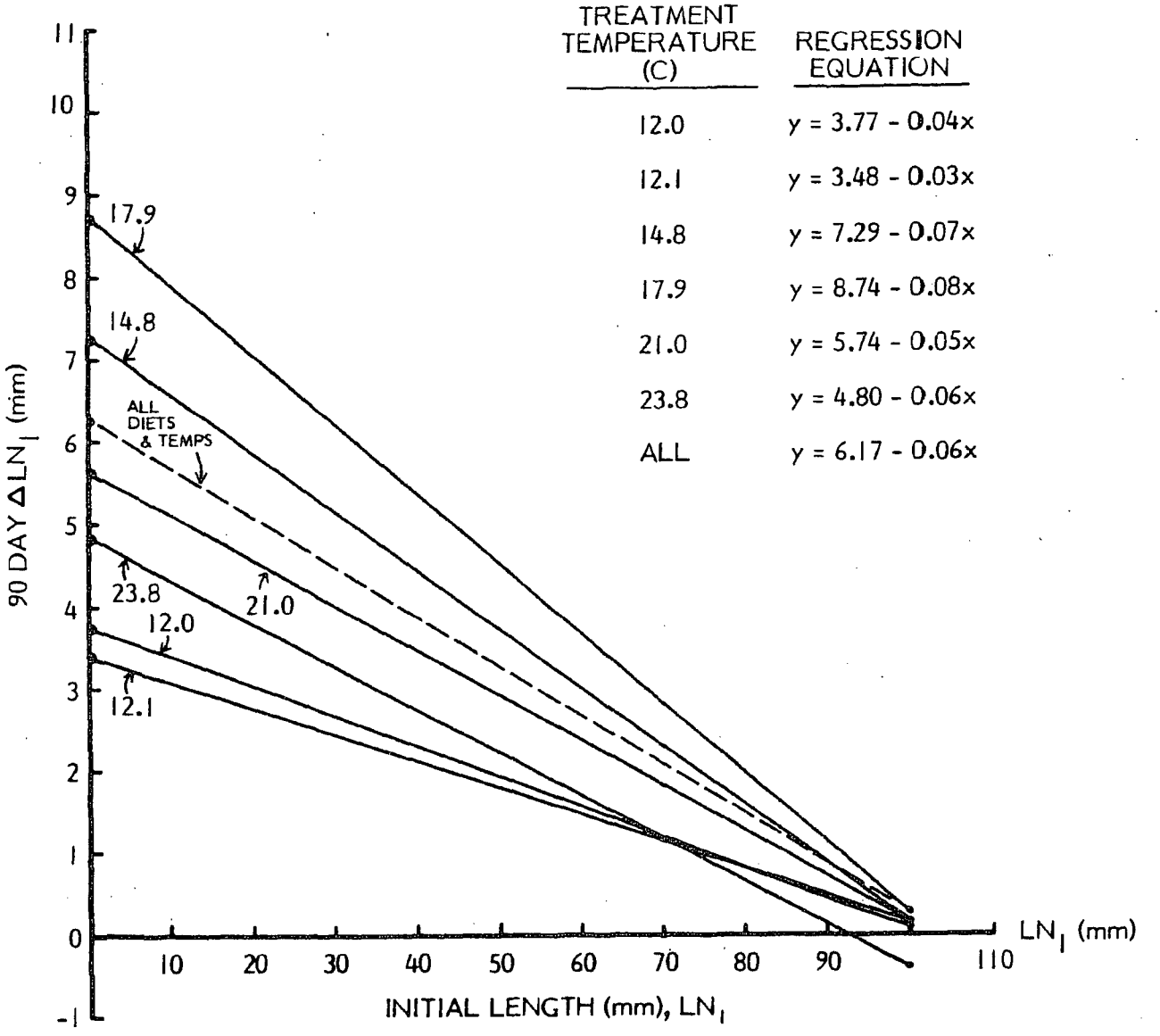


FIGURE 3.5-7

90 DAY CHANGE IN LENGTH VERSUS INITIAL LENGTH AT SIX TEMPERATURES WITH RED ALGAL DIET: BLACK ABALONE GROWTH

The dashed line is the regression for all treatments combined.

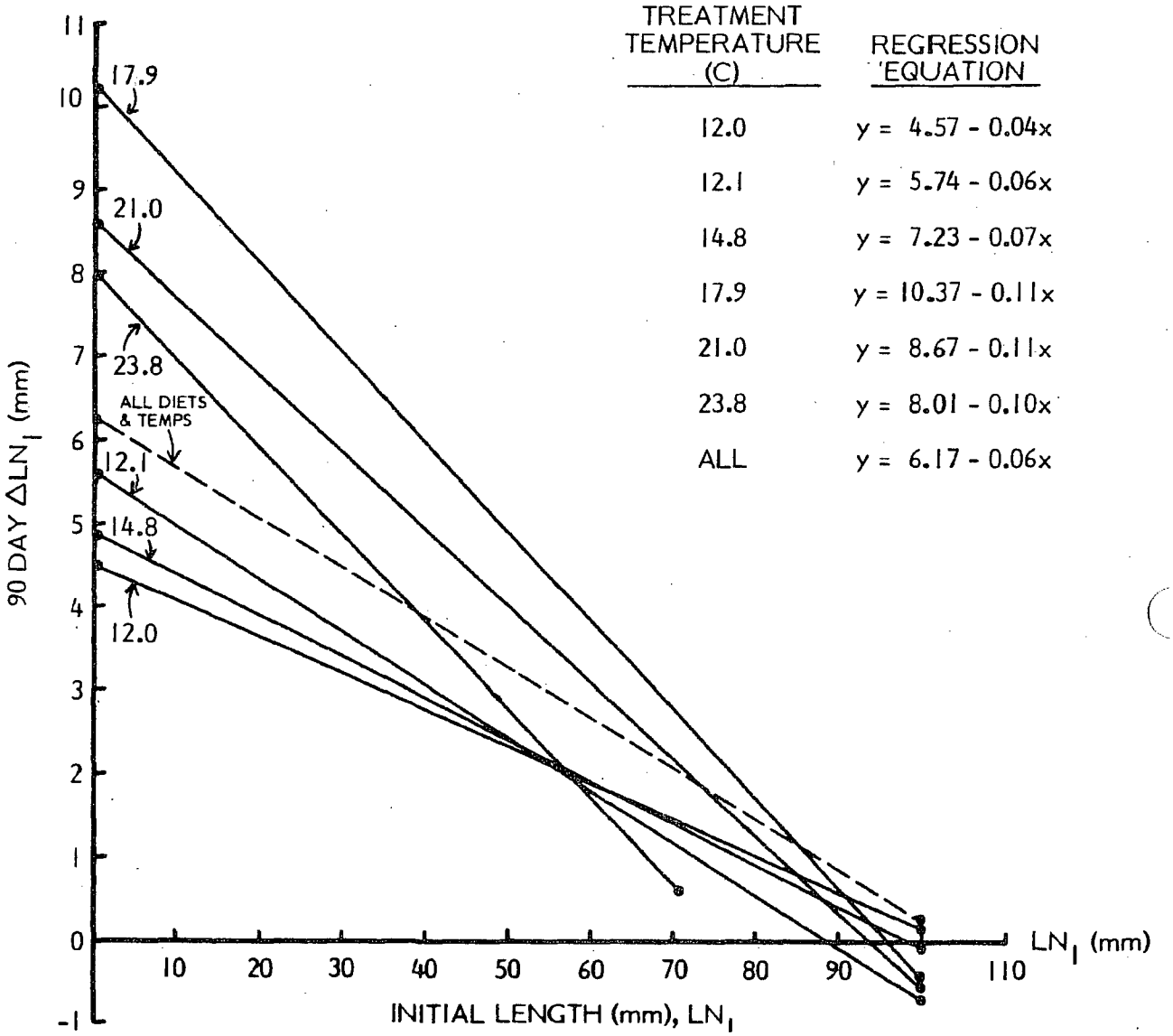


FIGURE 3.5-8

90 DAY CHANGE IN LENGTH VERSUS INITIAL LENGTH AT SIX TEMPERATURES WITH MIXED DIET: BLACK ABALONE GROWTH

The dashed line is the regression for all treatments combined.

3.5.2.3 DISCUSSION

Two studies of adult black abalone growth that have been reported include Wright (1975) and Leighton and Boolootian (1963). Wright's findings are based on a population of 74 animals from Santa Cruz Island, California. The abalone were measured at varying intervals for varying periods of time up to a year. Her data were transformed to 90-day mean growth rate by size class (from her Figure 4) and regressed (ΔLN vs LN_1) as shown in FIGURE 3.5-9. Similar transformations of Leighton and Boolootian's (1963) data were also performed from their Table V. Their data were based on two wild populations: 16 animals from Palos Verdes and 16 animals from Pt. Dume on the southern California coast. The resulting growth curves based on a regression of ΔLN vs LN_1 for a 90-day average are also shown in FIGURE 3.5-7. Their Palos Verdes population exhibited an unusual positive relationship between size of abalone and growth rate. However, inspection of their data revealed that the Palos Verdes population was composed of significantly smaller abalone than the Pt. Dume population (Palos Verdes mean = 47.1 mm, max LN_1 = 71.2 mm, min LN_1 = 28.0; Pt. Dume mean = 78.2 mm, max LN_1 = 125.3 mm, min LN_1 = 34.6 mm). Their conclusions on the comparative health of these populations based on growth rates would appear invalid on the basis of unequal size distribution in the two population samples.

The combined 90-day growth of all abalone (452) which were used in our laboratory growth studies are illustrated in FIGURE 3.5-10. The resulting regression equation from these data is also included in FIGURE 3.5-10 and added for comparison to FIGURE 3.5-9 illustrating Wright's (1975) and Leighton and Boolootian's (1963) growth results. A visual inspection of the line slopes indicates similar growth rates between our results and those of Wright (1975). The difference between these two slopes is attributed to differences in size frequencies in the two populations (temperature differences may also contribute to the different growth rates). Our sample contained a higher frequency of smaller abalone which, due to their faster growth rate, resulted in a regression coefficient larger than Wright's. Leighton and Boolootian (1963) apparently

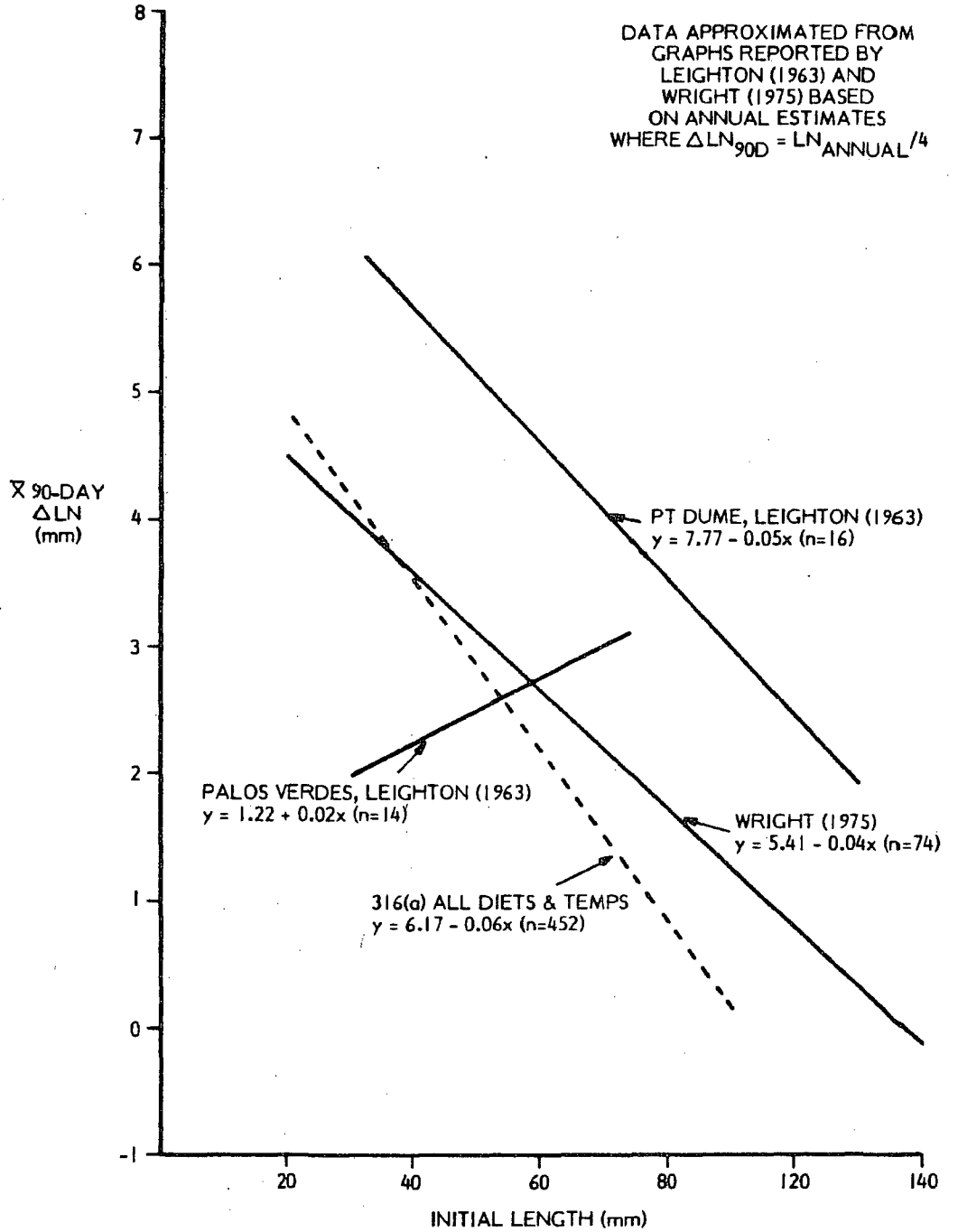


FIGURE 3.5-9

CHANGE IN LENGTH VERSUS INITIAL LENGTH
DATA FROM PREVIOUSLY PUBLISHED STUDIES
COMPARED WITH 316(A) STUDIES: BLACK ABALONE GROWTH

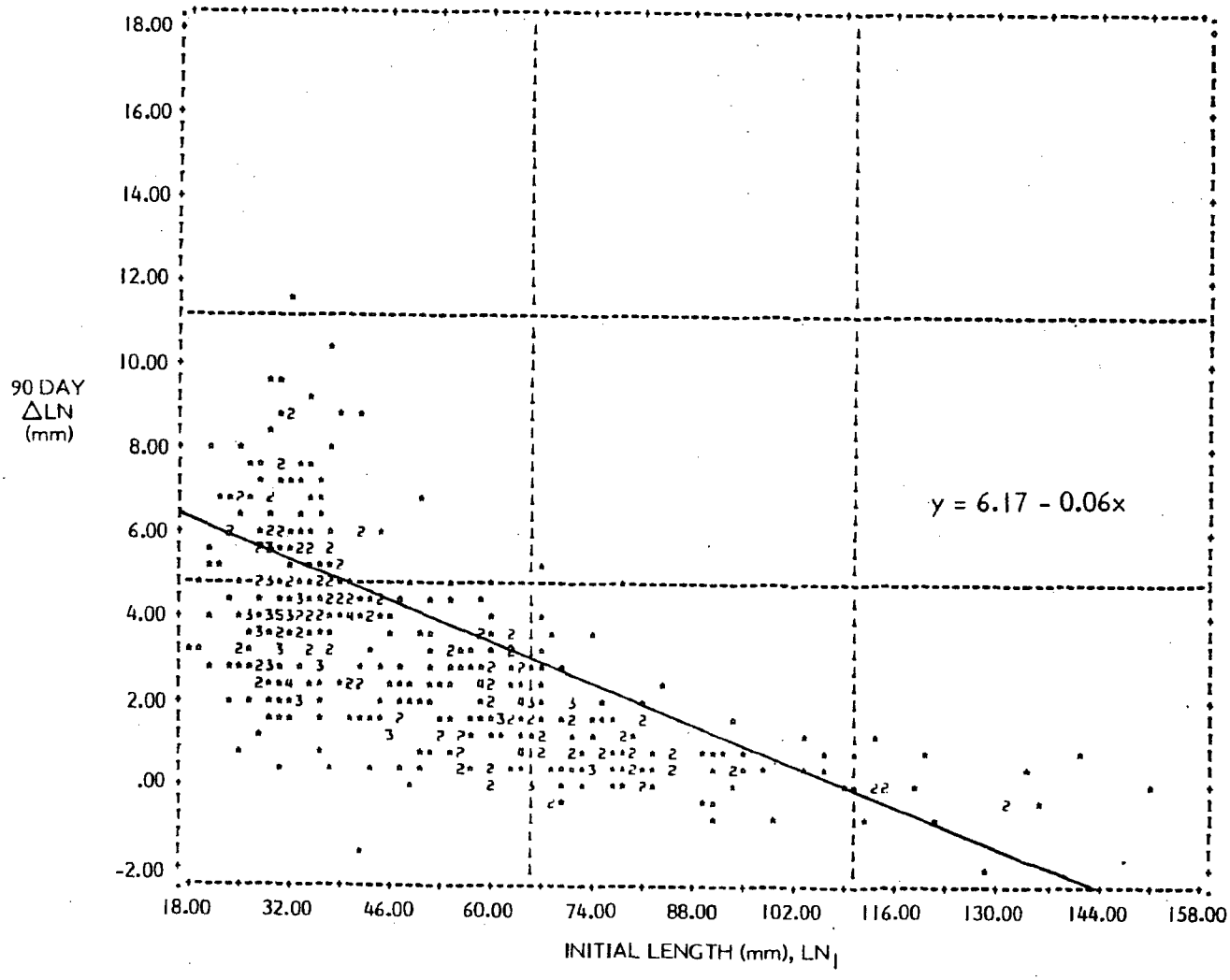


FIGURE 3.5-10

CHANGE IN LENGTH AT 90 DAYS VERSUS INITIAL LENGTH FOR ALL TEMPERATURE AND DIET TREATMENTS: BLACK ABALONE GROWTH

measured a population of abalone at Pt. Dume with considerably higher growth rates.

The effect of temperature on black abalone growth rates in our experiments were significant in the tested range of 12.0 to 23.8 C (53.6-74.8 F). Optimum growth occurred at or near 18 C (64 F). This temperature was slightly above the annual average maximum temperatures in Diablo Cove, but probably close to the average maximum temperature for the center of the black abalone population in southern California. Significant impairment of growth rates in the Diablo Cove populations were detected at the test temperaure 23.8 C (74.8 F). This temperature is approaching the measured 96-hour ET50 value of 27.4 C (81.3 F) for 16 C (60.8 F) acclimated animals from Diablo Cove area (PGandE 1978).

The effect of the three test diets on the black abalone growth rates was not significant at the 90 percent confidence level. However, trends in the results suggest that the brown and mixed brown and red algal diets sustained higher growth rates than the red algal diet. Leighton and Boolootian (1963) also reported lower growth potential for red algal species than for brown algal species. The brown and mixed red and brown algal diet produced similar growth rates in our study. During feeding in our experiments, the abalone which were fed a mixed diet consistently consumed the brown algal portion first, and it is assumed that this portion constituted the majority of their dietary intake.

3.6 RED ABALONE (HALIOTIS RUFESCENS)

The red abalone (Haliotis rufescens) is an RIS for the Diablo Canyon 316(a) demonstration. This species, historically important as a major commercial and sportfishery species, is primarily distributed in the subtidal region in the vicinity of Diablo Canyon (Burge and Schultz 1973). A detailed review of the taxonomy, ecology, life history and thermal biology of the red abalone was presented by PGandE (1979b). To orient the reader, a brief summary of the pertinent aspects of the biology of the red abalone is presented here.

Of the 75 species of worldwide abalone, the red abalone (Haliotis rufescens), is both the largest and the most commercially important. Its range extends from Sunset Bay, Oregon, to central Baja California. A common species along the California coast, the red abalone can be found from the high intertidal to depths of 540 feet (Cox 1960). In central California, where red abalone are the most abundant, they occupy a range from the low intertidal to depths of 30 feet (Cox 1962). Rarely encountered in sheltered bays, the red abalone is found predominately on rocky substrates exposed to moderate surf. Apparently this species requires considerable water movement in its habitat (McLean 1966). Abalone are selective plant feeders. Red abalone show distinct preferences for brown algae luetkeana. South of Point Conception, where Nereocystis is absent, they feed predominately on Macrocystis (Cox 1962).

Since the early 1900s the red abalone has been the subject of a large commercial and sport fishery. Prior to 1960 the commercial catch averaged between 2.4 and 2.8 million pounds per year (Cox 1960). This commercial effort has declined considerably in the last 15 years. Gotshall et al. (1977) attributes this decline to predation by an increasing sea otter population.

The results of three experiments are reported in this section. The first experiment studied the effects of temperature on fertilization of red abalone eggs and early development of embryos and larvae. The second experiment examined the effects of temperature on settlement of larvae, when individuals make the transition to the bottom dwelling phase. The effects of temperature

and diet on growth of juvenile red abalone was investigated in the third experiment.

3.6.1 FERTILIZATION AND EARLY DEVELOPMENT

The objective of this experiment was to study the effects of temperature on fertilization and early development in red abalone. A wide range of temperatures (10 to 26 C) was used so as to determine minimum, maximum and optimum temperatures or range for successful development. The purpose was to ascertain the possible effects of the power plant thermal discharge on recruitment and development of red abalone in Diablo Cove.

A large body of literature exists on development in haliotids (Ino 1952, Leighton 1972 and 1974, Morse et al. 1977 and 1979a, b, Murayama 1935, Oba 1964, and Crofts 1937). Research dealing specifically with the red abalone has been published by Leighton (1974) and Morse et al. (1977 and 1979a, b). Leighton's work deals directly with temperature and its effect on larval development. The major portion of his work deals with posthatch and juvenile stages. This experiment was designed to determine the effects of temperature on the embryonic and larval stages.

3.6.1.1 MATERIALS AND METHODS

A summary description of the methods employed in the investigations into the effects of temperature on development in red abalone is presented below. For a more detailed account of the procedures used see APPENDIX B (Procedure 324) and APPENDIX C (protocol for experiment 24a). The incubation gradient apparatus used in this experiment is described in APPENDIX A.

Pre-experimental

Mature male and female red abalone were obtained from the power plant intake cove using SCUBA equipment. All animals were held at ambient temperature in

the laboratory for a period of two weeks prior to spawning. During this time the animals were fed Macrocystis to excess.

Groups of gravid male and female abalone were spawned in separate tubs using the Tris-H_2O_2 method as described by Morse et al. (1977). The spawn was used to fertilize a fertilization experimental group, a postcleavage experimental group, a posthatch experimental group and an ambient control group. Following fertilization the eggs were washed repeatedly to remove any excess sperm. The two groups were placed in 2-liter beakers with 0.2 μm filtered UV sterilized seawater at ambient temperature.

Experimental

The fertilization group consisted of a series of test tubes, one for each temperature treatment, and each containing approximately 150 fertilized eggs. Each test tube was placed in a styrofoam block suspended in seawater at ambient temperature. The beaker was then transferred to the appropriate chamber in the incubation gradient. After 12 hours the test tubes were removed and all samples were preserved in one percent formalin solution for later observation on the number of noncleaved and abnormal eggs and the number of eggs at each developmental stage.

The postcleavage group consisted of treatment populations produced by separate fertilizations at half-hour increments and placed into the treatment chambers in the gradient in a predetermined random order at half-hour increments to allow time for sampling all treatments at identical points in time following initiation of the treatment conditions. After a 4-hour incubation period at ambient temperature three replicates of 50 cleaved embryos were placed in three test tubes and transferred to a 2-liter beaker filled with seawater initially at ambient temperature in the appropriate sector of the incubation gradient. This was done for all eight test temperatures. At 4 and 12 hours post-treatment, observations were made on all replicates for mortalities and developmental stage. At 28, 52 and 76 hours post-treatment, one of the test tubes was removed from each

treatment, treated with the relaxant MS222 and preserved in one percent formalin for later observation.

The posthatch group was transferred to the incubation gradient following hatching (approximately 24 hours after fertilization) from the group set aside at the initial fertilization. Three replicates of 50 swimming trochophores each were placed into three test tubes per treatment, and then transferred into the incubation gradient. One replicate was removed from each treatment at 12, 36 and 60 hours post-treatment, relaxed in MS222 and preserved in one percent formalin for later observation.

Observations on the three groups at the eight test temperatures (10, 12, 16, 18, 20, 22, 24 and 26 C) included numbers of larvae at each developmental stage and number of abnormalities or mortalities. Due to infection of samples by ciliated protozoans and bacteria, which became more pronounced with time, only the results from the 4-, 12- and 28-hour observations for the postcleavage group and the 12-hour observation for the posthatch group are reported.

3.6.1.2 RESULTS

A summary and analysis of all temperature, development and mortality data collected during the experiment are included in this section.

Temperature Data

The temperatures given in TABLE 3.6-1 are summarized for the entire experiment (1400 hours, 4 February 1980 to 0600 hours, 8 February 1980). Although there are 20 compartments in the incubation gradient, only eight were used as actual test compartments, as indicated in TABLE 3.6-1. All other references to temperature in this section are to the mean values presented for the eight experimental treatments.

TABLE 3.6-1

SUMMARY OF TEMPERATURE DATA:
RED ABALONE LARVAL DEVELOPMENT

Autodata Nine Channel	Actual Temperatures (C)		Target Temperatures (C)*
	Cumulative Mean	Standard Deviation	
91	4.5	0.2	
92	5.8	0.2	
93	8.0	0.2	
94	8.6	0.3	10
95	11.2	0.6	
96	12.0	0.4	12
97	12.9	0.4	
98	13.8	0.5	
99	14.9	0.2	
100	16.5	0.2	16
101	17.6	0.2	
102	18.6	0.3	18
103	18.9	0.6	
104	20.0	0.4	20
105	20.8	0.4	
106	22.4	0.5	22
107	23.3	0.2	
108	24.8	0.2	24
109	26.1	0.2	26
110	27.9	0.3	

* Target temperatures shown for compartments used in this experiment.

Survival

Survival in the various treatment combinations was measured as the percentage of larvae in the sample undergoing normal development. Any abnormal larvae were scored as mortalities. Proportions of survival in each sample at 12 hours post-treatment are presented for the three experimental groups in TABLE 3.6-2. Proportions of survival in the postcleavage group at 4, 12 and 28 hours are presented in TABLE 3.6-3. A comparison of percentage survival at 12 hours for the three test groups is presented in FIGURE 3.6-1. A chi-square test for independence was performed on the data from the three groups resulting in a computed χ^2 value of 289.64. Therefore, the hypothesis of independence at all x-values χ^2 (α = 0.005, d.f. = 14) = 31.32 is rejected and it is concluded that observed changes in percentage survival with temperature are dependent upon the group from which the percentages were obtained.

The data were then tested using the method of Sokal and Rohlf (1969). An example of the computation and the results is presented in TABLE 3.6-4. These substantiate what is shown in FIGURE 3.6-1, i.e., temperature ranges for survival are most restrictive in larvae undergoing cleavage, less restrictive in the postcleavage stages and least restrictive in larvae transferred to temperature following hatching. The multiple groupings for the posthatch group are a result of the sensitivity of this test. Ninety-five percent confidence intervals on the percentages would result in fewer groupings due to the smaller sample size in this group.

Curves of survival with time for the postcleavage group are presented in FIGURE 3.6-2. A chi-square test for independence was performed on the percentages at the eight temperatures for the three observations resulting in a computed χ^2 value of 302.80 which is significant at all x-values χ^2 (α = 0.005, d.f. = 14) = 31.32 . The hypothesis of independence is therefore rejected and it can be concluded that survival at different temperatures is dependent upon the time the observations are taken. A test for differences between treatments was performed only for the 28-hour observation because pronounced differences in survival were observed only at that time. Using the same formula presented in

TABLE 3.6-2

PROPORTIONS OF SURVIVAL IN THREE GROUPS OF LARVAE FOLLOWING 12 HOURS EXPOSURE TO TREATMENT: RED ABALONE LARVAL DEVELOPMENT

Mean Temperature (C)	Experimental Group		
	Fertilization	Postcleavage*	Posthatch
8.6	0.148	0.987	0.87
12.0	0.835	0.993	1.00
16.5	0.813	0.722	0.905
18.6	0.860	0.994	0.540
20.0	0.853	1.000	0.280
22.4	0.067	0.993	0.708
24.8	0.006	0.007	0.563
26.1	0.009	0.000	0.162

* Values for three replicates were pooled.

TABLE 3.6-3

PROPORTIONS OF SURVIVAL AT 4, 12 AND 28 HOURS
FOR THE POSTCLEAVAGE GROUP:
RED ABALONE LARVAL DEVELOPMENT

The values result from pools of three replicates at
4 and 12 h and for one replicate at 28 h.

Mean Temperature (C)	Sample Time (h)		
	4	12	28
8.6	0.987	0.987	0.777
12.0	0.974	0.993	1.000
16.5	0.965	0.722	0.621
18.6	0.993	0.994	0.823
20.0	0.962	1.000	0.565
22.4	0.993	0.993	0.372
24.8	0.971	0.007	0.000
26.1	0.974	0.000	0.000

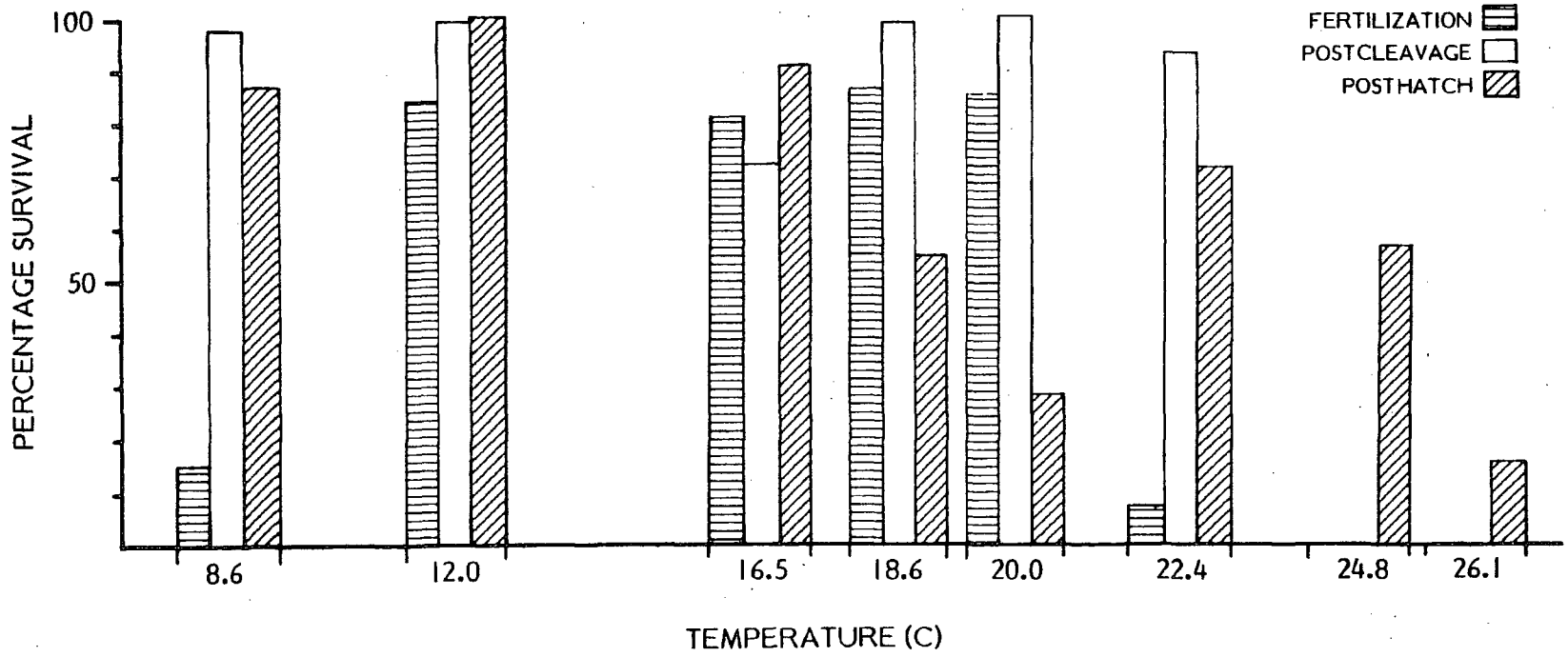


FIGURE 3.6-1

PERCENTAGE SURVIVAL VERSUS TEMPERATURE
AT 12 HOURS: RED ABALONE
LARVAL DEVELOPMENT

TABLE 3.6-4

TEST OF SIGNIFICANCE OF SURVIVAL DATA
AT 12 HOURS BETWEEN TREATMENTS:
RED ABALONE LARVAL DEVELOPMENT

(Treatments connected by underline are not significantly different.)

Statistics computed according to Sokal and Rohlf (1969) as follows:

$$t_s = \frac{\arcsin \sqrt{P_1} - \arcsin \sqrt{P_2}}{\sqrt{820.8\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$$

at $\alpha = 0.99$, d.f. = ∞.

Experimental Group	Treatment (C)							
Fertilization	18	20	12	16	10	22	26	24
Postcleavage	20	18	22	12	10	16	24	26
Posthatch	12	16	10	22	24	18	20	26

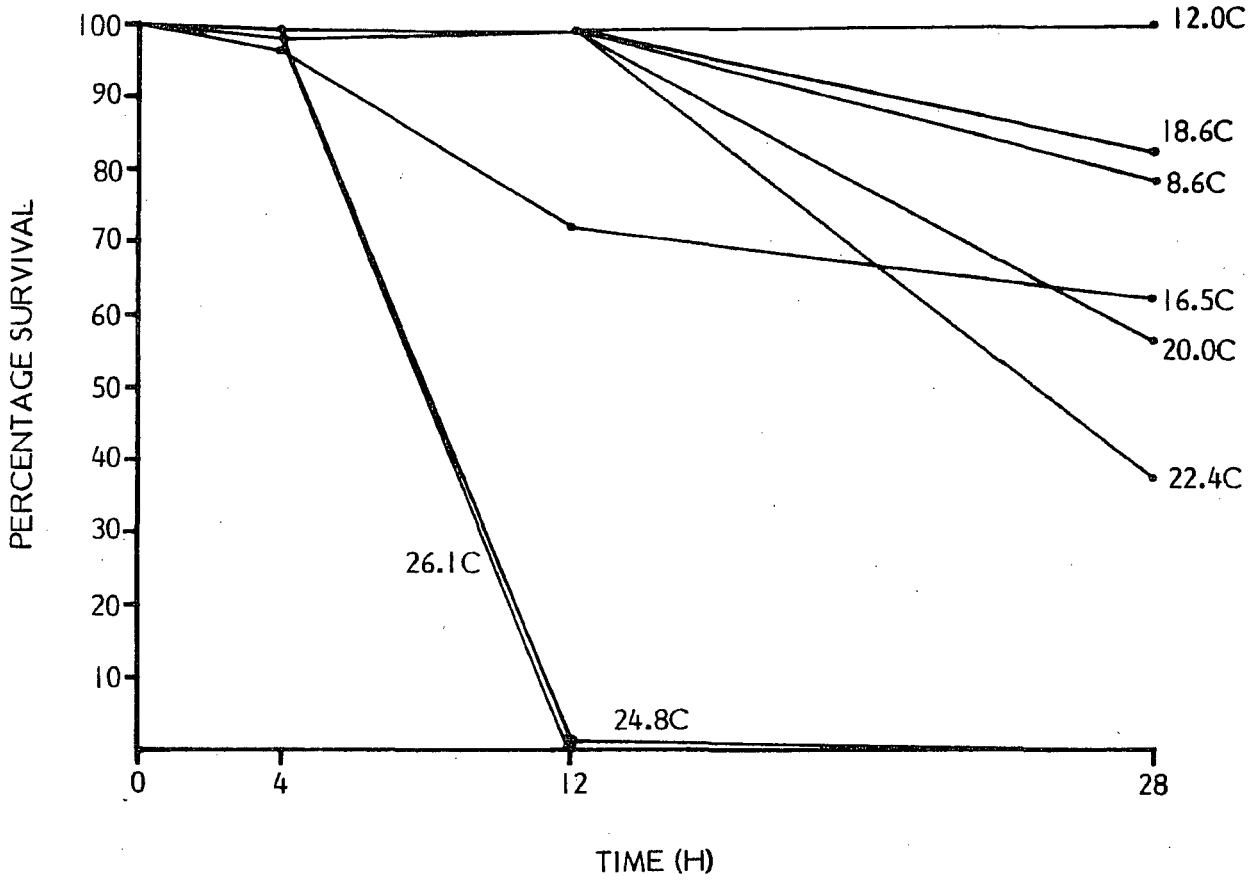


FIGURE 3.6-2

PERCENTAGE SURVIVAL VERSUS TIME
FOR THE POST CLEAVAGE GROUP: RED ABALONE
LARVAL DEVELOPMENT

TABLE 3.6-4, with t, $\alpha = 0.01$, d.f. = ∞, = 2.576, the following groupings of the treatments were obtained:

- o 12 18 10 16 20
- o 22

These accord well with the separations revealed in FIGURE 3.6-2.

At 12 hours survival was essentially zero in the 24.8 and 26.1 C treatments. Of the remaining treatments, only the 16.5 C treatment showed a decrease in survival at 12 hours. Considering that no decrease in survival was observed at this time in three other treatments up to 22.4 C, the decrease at 16.5 cannot result from thermal stress and this anomalous result is unexplained. By 28 hours (see FIGURE 3.6-2) there were marked differences in survival at the different temperatures; however, only the 22.4 C treatment was statistically different from the other treatments, as shown above.

Development

Development was examined in the different treatment combinations by staging the larvae. Development was fairly synchronous within replicates (when present) for the different treatment combinations and within individual samples. The results of the staging at 12 hours for the fertilization, postcleavage and posthatch groups are presented in TABLE 3.6-5. Development at 4, 12 and 28 hours for the postcleavage group is presented in TABLE 3.6-6. Development in the fertilization group at 12 hours for all treatments is shown in FIGURE 3.6-3. Greater than 95 percent mortality evidenced by lack of cleavage or abnormality occurred at 22.4 C and above. "Normal" fertilization success might be postulated to be about 80 to 85 percent as evidenced by the treatments between 12 and 20 C. In the 8.65 C treatment the unusually large proportion of abnormalities indicated that no development occurred at that temperature. It is difficult to determine whether this response resulted from thermal stress or could possibly have resulted from contamination of the glassware since even at

TABLE 3.6-5

STAGE OF DEVELOPMENT DATA AT 12 HOURS:
RED ABALONE LARVAL DEVELOPMENT

Data from three replicates were pooled to give the results for the posthatch group.

Experimental Group	Developmental Stage	TREATMENT TEMPERATURE (C) 8.6	12.0	16.5	18.6	20.0	22.4	24.8	26.1
Fertilization									
	2-cell Embryo	10					9	1	2
	4-cell Embryo	7					3		
	8-cell Embryo	4							
	16-cell Embryo	2							
	Morula		212				4		
	Early Gastrula		6	156					
	Rotating Gastrula			8	197	55			
	Prehatch Trochophore				54	184			
	Swimming Trochophore								
Postcleavage									
	2-cell Embryo				0, 1, 0				
	4-cell Embryo	0, 0, 1			0, 1, 0	1, 0, 3			
	8-cell Embryo					1, 0, 1			
	16-cell Embryo							0, 1, 0	
	Morula	4, 2, 0	49, 0, 0						
	Early Gastrula	52, 42, 46	0, 49, 51	35, 36, 0	48, 55, 50				
	Rotating Gastrula			4, 0, 39	1, 0, 1	51, 53, 58			
	Prehatch Trochophore					0, 0, 5	46, 48, 49		
	Swimming Trochophore						0, 0, 2		
Posthatch									
	Swimming Trochophore	28							
	Cap-shell Veliger	11							
	Inflate-shell Veliger								
	Opercolate Veliger		49	38	22				6
	Incipient Cephalic Tentacle Veliger					21	51	36	

TABLE 3.6-6

STAGE OF DEVELOPMENT FOR THE POSTCLEAVAGE GROUP AT 4,12 AND 28 HOURS: RED ABALONE LARVAL DEVELOPMENT

Data from three replicates except one replicate for the 28h sample.

Observation (h)	Developmental Stage	TREATMENT TEMPERATURE (C) 8.6	12.0	16.5	18.6	20.0	22.4	24.8	26.1
	2-cell Embryo	0, 0, 1			0, 1, 0			1, 0	
	4-cell Embryo	0, 0, 2		0, 1, 0	0, 1, 0	1, 0, 0			0, 1, 0
4	8-cell Embryo	1, 3, 8		0, 1, 0		0, 0, 1			5, 3, 0
	16-cell Embryo	50, 43, 39	38, 44, 52	4, 1, 1	2, 1, 0	0, 30		1, 13	45, 44, 48
	Morula		12, 6, 0	48, 49, 57	30, 32, 30	23, 21, 28		47, 37	0, 0, 2
	Early Gastrula			1, 1, 1	18, 18, 19	23, 28, 23	48, 44, 51		
	2-cell Embryo				0, 1, 0				
	4-cell Embryo	0, 0, 1			0, 1, 0	1, 0, 3			
	8-cell Embryo					1, 0, 1			
12	16-cell Embryo							0, 1, 0	
	Morula	4, 2, 0	49, 0, 0						
	Early Gastrula	52, 42, 46	0, 49, 51	35, 36, 0	48, 55, 50				
	Rotating Gastrula			4, 0, 39	1, 0, 1	51, 53, 58			
	Prehatch Trochophore					0, 0, 5	46, 48, 49		
	Swimming Trochophore						0, 0, 2		
	Swimming Trochophore	7	44	7	5	2	2		
	Cap-shell Veliger			2		6			
28	Inflate-shell Veliger			9	7	5	14		
	Operculate Veliger				2				

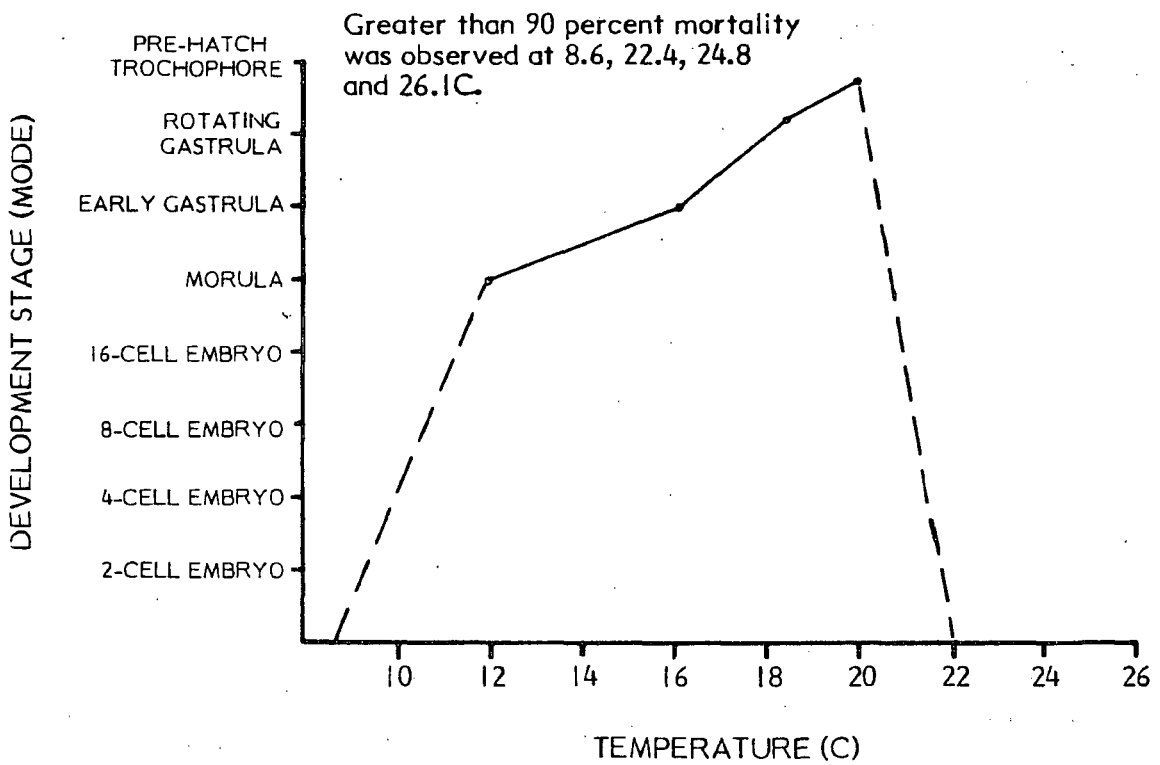


FIGURE 3.6-3

STAGE OF DEVELOPMENT VERSUS TEMPERATURE FOR THE FERTILIZATION GROUP AT 12 HOURS: RED ABALONE LARVAL DEVELOPMENT

the higher temperatures (22 to 26 C) temperature effects on development were evidenced by a lack of cleavage, rather than a high percentage of abnormalities.

Development in the postcleavage group for the eight temperature treatments over time is presented in FIGURE 3.6-4. Observations at 52 and 76 hours on this group are not included due to contamination of the samples by bacteria and ciliated protozoans. Greater than 95 percent mortality occurred in the 24.8 and 26.1 C treatments following the 4-hour observation. Development in the range 8.6 to 18.6 C seemed fairly synchronous over time. An increased rate of development occurred at 20.0 and 22.4 C.

Results on development in the posthatch group are presented only for the 12-hour observation. Predation by ciliated protozoans appreciably reduced the sample size in later observations. As is shown by FIGURE 3.6-5, development following hatching is severely retarded at 8.6 C. Within the range 12 to 24 C development appeared normal with little variation between treatments up to 12 hours following exposure to the test temperatures.

3.6.1.3 DISCUSSION

Based on the results of this experiment, fertilization and initial cleavage of red abalone eggs is successful from 12 to 20 C (54 to 68 F). At 8 C (46 F) and 22 C (72 F) and above, fertilization success is very low. Survival of the embryonic stages occurs over a somewhat broader range, from 8 to 22 C (46 to 72 F) with essentially no survival at 24 C (75 F) and above. Survival of the larval stages occurs over a somewhat broader range than the embryonic stages, ranging from 8 to 24.8 C (46 to 76.6 F). About 50 percent survival was observed at 24.8 C (76.6 F) and a much lower survival was observed at 26 C (79 F). Rates of development of all groups (fertilization, embryonic and larval) increased with increasing temperature up to the lethal temperature for that stage of development. These results, which agree with those of Leighton (1974), show that the cleavage and gastrulation processes are the most sensitive of the early development stages of the red abalone to thermal stress.

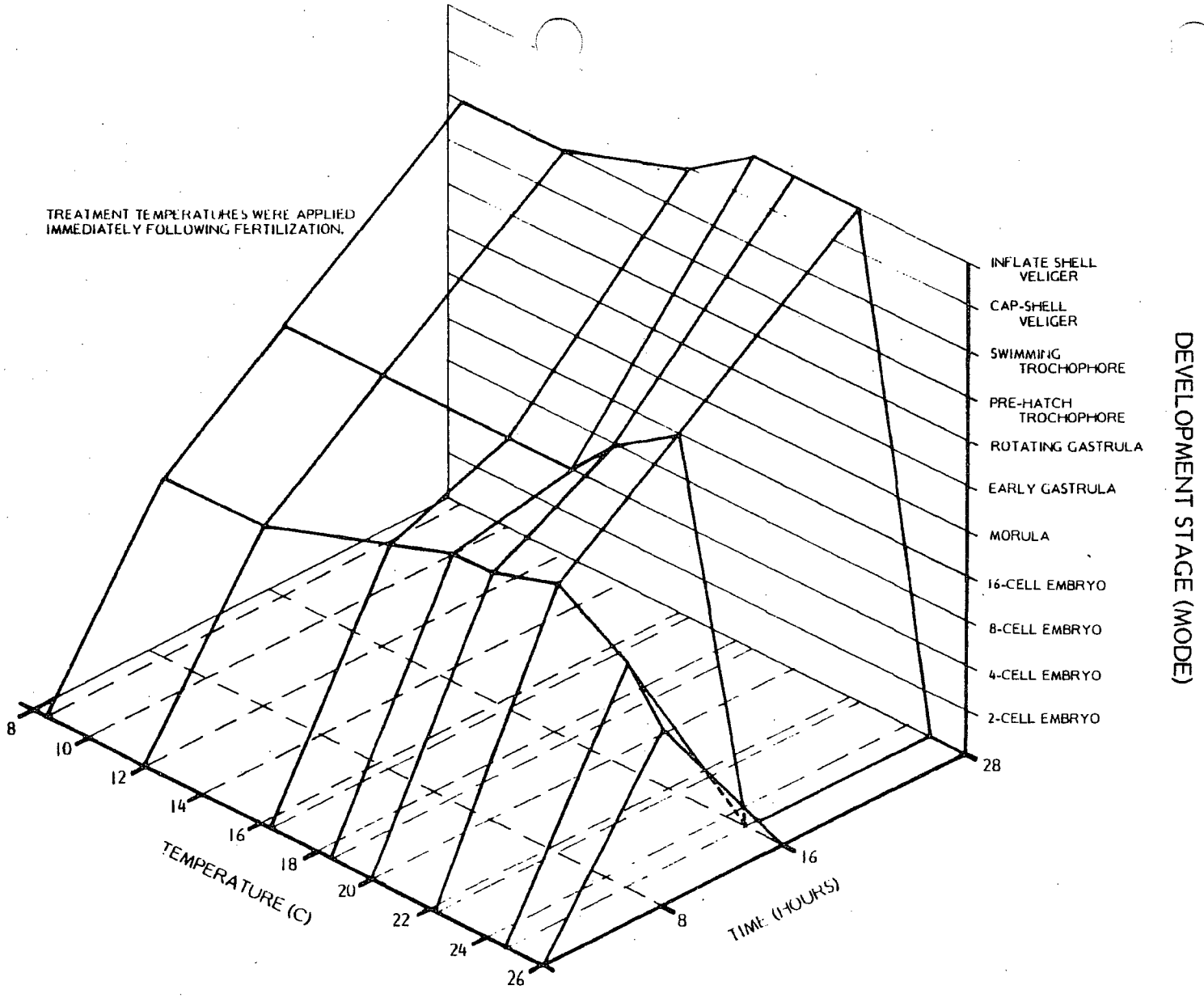


FIGURE 3.6-4

DEVELOPMENT STAGE VERSUS
TEMPERATURE AND TIME FOR THE POST CLEAVAGE GROUP:
RED ABALONE LARVAL DEVELOPMENT

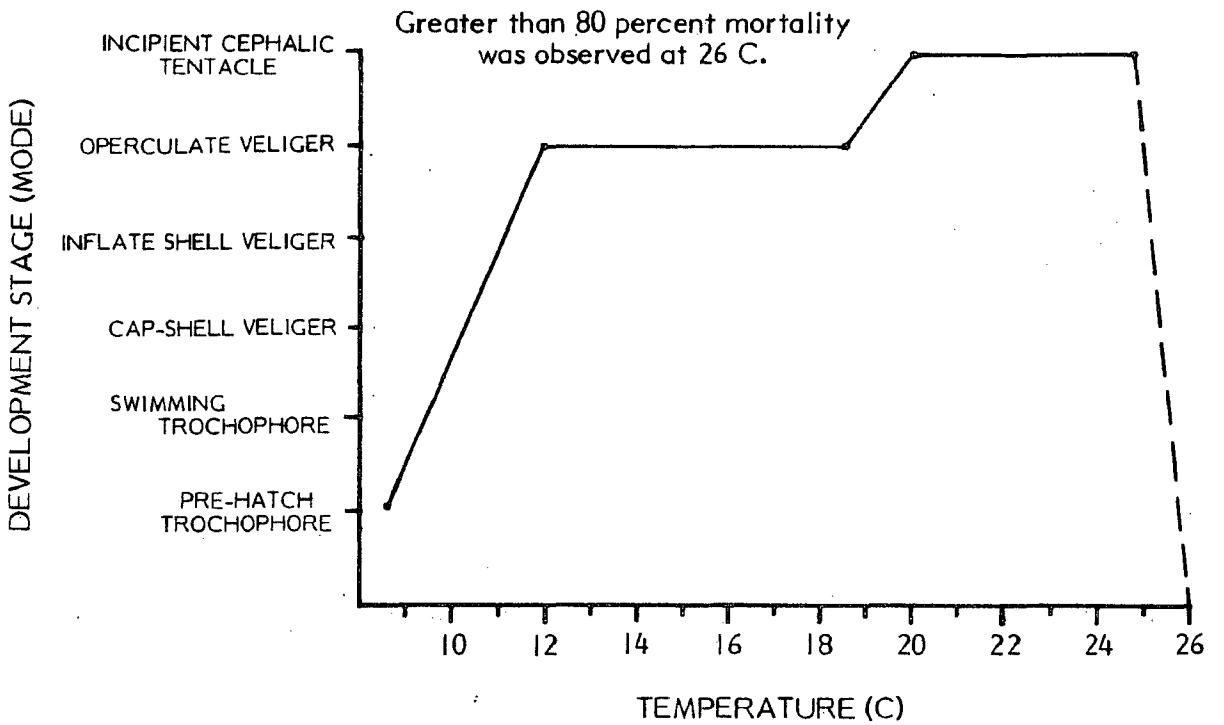


FIGURE 3.6-5

STAGE OF DEVELOPMENT VERSUS
TEMPERATURE FOR THE POSTHATCH GROUP:
RED ABALONE LARVAL DEVELOPMENT

3.6.2 LARVAL SETTLEMENT

In the previous section the results of an experiment on red abalone larvae to determine the effects of temperature on larval development and survival were reported. The physiological processes of zygote and embryonic development and hatching were included in those stages of development examined. The process of early development terminates when the planktonic free-swimming larvae settle onto hard substrate and undergo metamorphosis into the juvenile bottom dwelling stage. The effect of temperature on this final step in early development was studied in the experiment reported in this section.

A brief description and review of thermal tolerance studies of red abalone were included in the previous section. The study objective of this experiment was to measure under laboratory conditions the settlement success of red abalone larvae at different temperatures and the potential of using GABA (γ-amino-butyric acid) in the bioassay. The purpose was to assess the possible effects of the power plant thermal effluent on recruitment and settlement of red abalone in Diablo Cove.

3.6.2.1 MATERIALS AND METHODS

The following description of materials and methods employed in the settlement experiments on red abalone summarizes the key points of the experimental design and methodology. For a more detailed account of the laboratory methods used to study the effects of temperature on settlement in red abalone, see APPENDIX B (Procedure 323) and protocol, APPENDIX C (experiment 23a).

The experimental design called for exposure of trochophore larvae (determined to have settlement competency) to a range of temperature treatments. Observations were made on the number of settled, nonsettled and dead abalone larvae in each treatment at three observation times. Settlement was defined by snail-like movement of the foot, loss of velum, and growth of adult peristomial shell. Death was defined by an empty shell or an overturned shell with total lack of movement. A total of 32 treatments, incorporating eight temperatures (10, 12,

16, 18, 20, 22, 24 and 26 C) and three concentrations of GABA (1×10^{-6}, 3×10^{-6} and 6×10^{-6} M) and a control with no GABA were tested. Two replicates were used in each treatment.

Pre-experimental

Mature male and female red abalone were collected from the power plant intake cove at Diablo Canyon. The abalone were held for two weeks prior to spawning, during which time they were fed Macrocystis to excess. These animals were spawned using H_2O_2-Tris, as described by Morse et al. (1977). Eggs were fertilized and then washed repeatedly to remove excess sperm. Embryos were maintained until hatching at ambient temperature (14-15 C) in a 2-liter beaker. Following hatching the swimming trochophore larvae were siphoned off and placed into a flow-through system using 3 m-filtered, U.V. sterilized seawater. The larvae were maintained in this manner until a stage competent for settlement had been reached.

Experimental

After eight days the larvae were transferred to a 2-liter beaker at a final density of approximately 10 larvae/ml. Antibiotics (penicillin-G and streptomycin-sulfate) were added in a concentration of 150 mg/l at 0.5-hour intervals to minimize bacterial contamination. For each treatment, eight shell vials were inoculated with 9 ml of the larval suspension, stoppered, and placed in a rack suspended in a tray of ambient seawater. The tray was then transferred to a 4 by 8 ft flow-through tank programmed to the specified test temperature. This procedure was carried out for one treatment every 0.5 hour until all treatments were set up. After a 4-hour acclimation period, a 1 ml aliquot of GABA was added to each test vial at concentrations calculated to result in the specified treatment concentration after dilution in the vial. The control vials received 1 ml of sterile water at this point to provide for equal density in all vials.

Observations were made at 12, 40 and 90 hours after addition of GABA. These took place at 0.5-hour intervals to coincide with the staggered experimental

startup and GABA additions. Observations were made by placing the vial under a dissecting microscope, taking care to minimize temperature excursions by keeping the vials in a water bath except during actual counting. The number of settled, nonsettled and dead larvae were counted in each vial. Total numbers were calculated after the 90-hour observation by adding formalin to each vial to a final concentration of 1 percent. Using the total sample size in each vial counted at this point, proportions of settled and dead larvae were calculated. Qualitative observations on condition, abnormalities and presence of peristomal shell were also taken throughout the experiment.

Temperatures

Hourly temperatures were recorded on paper tape by the Autodata Nine computer. These data were used to calculate daily means and standard deviations, as shown in TABLE 3.6-7.

3.6.2.2 RESULTS

This experiment was initiated on 14 and terminated on 17 March 1980. Mortality and settlement data are expressed as a proportion of the total number of larvae counted in each vial at the end of the experiment. From preliminary analyses of the data, by examination of residuals, the following transformation was determined to satisfy all statistical criteria and was used for all statistical procedures:

$$\sin^{-1} \sqrt{(\text{proportion})}$$

Mortality

Mortality due to predation by ciliated protozoans was observed in the control vials at all temperatures. The level of mortality ranged from a mean low of 15 percent at 20 C to a mean high of 35 percent at 12 C (TABLE 3.6-8). Using a one-way ANOVA on the transformed data, no significant differences between temperatures were found.

TABLE 3.6-7

SUMMARY OF TEMPERATURE DATA:
RED ABALONE LARVAL SETTLEMENT

(Means of hourly readings are given with standard deviations in parentheses).

Date (March 1980)	Target Temperature (C)							
	10	12	16	18	20	22	24	26
13	10.8 (0.67)	12.0 (0.0)	16.0 (0.0)	18.0 (0.0)	19.9 (0.0)	21.9 (0.0)	19.8 (0.4)	26.0 (0.0)
14	10.3 (0.2)	12.0 (0.2)	16.0 (0.0)	18.0 (0.0)	19.9 (0.0)	21.9 (0.2)	22.6 (2.2)	26.0 (0.1)
15	10.3 (0.1)	12.0 (0.0)	16.0 (0.0)	18.0 (0.0)	19.9 (0.0)	21.8 (0.1)	24.0 (0.0)	26.0 (0.1)
16	10.2 (0.1)	12.0 (0.0)	16.0 (0.0)	18.0 (0.0)	19.8 (0.1)	21.7 (0.1)	24.0 (0.0)	26.0 (0.1)
17	10.3 (0.1)	12.0 (0.0)	16.0 (0.0)	18.0 (0.0)	19.8 (0.0)	21.7 (0.1)	24.0 (0.0)	26.0 (0.1)

TABLE 3.6-8

PROPORTIONS OF MORTALITIES IN CONTROLS AT 40 HOURS:
RED ABALONE LARVAL SETTLEMENT

Replicate	Target Temperature (C)							
	10	12	16	18	20	22	24	26
A	0.21	0.35	0.23	0.21	0.15	0.26	0.21	0.28
B	0.20	0.35	0.23	0.21	0.15	0.13	0.13	0.23

ANOVA

Source	Degrees of Freedom	Sums of Squares	Mean Squares	F Value
Treatment	7	26.68	3.811	0.107*
Error	8	285.43	35.68	
Total	15	312.11		

* Not significant.

A tabulation of proportions of larval mortalities for all treatment vials is presented in TABLE 3.6-9. The mean proportions of mortalities are plotted against time and temperature for GABA concentrations of 1×10^{-6}M in FIGURE 3.6-6, 3×10^{-6}M in FIGURE 3.6-7, and 6×10^{-6}M in FIGURE 3.6-8.

Multiple regression analysis on the transformed mortality data at each concentration was performed and summaries are shown in TABLE 3.6-10. The mortality data fit a first order linear model, lacking any interaction terms which is in agreement with the appearance of the curves in FIGURES 3.6-6 to 3.6-8.

An analysis of covariance on the transformed mortality data, using "dummy" variables for the three GABA concentrations, was performed and the results are shown in TABLE 3.6-11. Analysis using a factorial design analysis of covariance was negated due to the significant interaction between treatments. Therefore, each GABA concentration was analyzed separately for differences between temperature treatments. The results of analyses of covariance for each GABA concentration is presented in TABLE 3.6-12. This analysis reveals significant differences between slopes at each temperature treatment. No significant differences between temperatures were demonstrated in the 1×10^{-6}M GABA treatments. Significant F-values were obtained for both the 3×10^{-6}M and 6×10^{-6}M GABA treatments. A priori comparisons were performed on the latter two groups and the results are given in TABLE 3.6-13.

From the a priori comparisons, two distinct groups were detected at the highest GABA concentration. At this concentration, a significantly higher mortality rate occurred at temperatures greater than 18 C. At 3×10^{-6}M GABA the two groupings were less distinct but two groups appear to have been partially defined. One group shows no significance from 10 to 20 C. A group composed of 24 and 26 C is also present. At this concentration temperatures greater than 20 C showed significantly higher mortality.

Differences between concentrations were tested using a two-way ANOVA on the 90-hour mortality values. Control values were included as a fourth treatment level. Using all temperature levels in the analysis, significant interaction occurs

TABLE 3.6-9

PROPORTIONS OF MORTALITIES:
RED ABALONE LARVAL SETTLEMENT

(Data for Replicates A and B are shown.)

Target Temperature (C)	GABA Concentration (x 10^{-6}M)	Observation Time (hours)					
		12		40		90′	
		A	B	A	B	A	B
10	1	0.00	0.00	0.00	0.04	0.39	0.31
	3	0.00	0.00	0.05	0.09	0.15	0.32
	6	0.00	0.00	0.00	0.02	0.28	0.28
12	1	0.00	0.00	0.02	0.00	0.26	0.22
	3	0.00	0.00	0.00	0.12	0.19	0.45
	6	0.00	0.00	0.00	0.00	0.50	0.32
16	1	0.00	0.00	0.18	0.09	0.18	0.22
	3	0.00	0.00	0.00	0.11	0.28	0.42
	6	0.00	0.00	0.12	0.00	0.37	0.32
18	1	0.00	0.00	0.10	0.09	0.41	0.39
	3	0.00	0.00	0.13	0.05	0.42	0.44
	6	0.00	0.00	0.08	0.05	0.55	0.55
20	1	0.00	0.00	0.16	0.11	0.34	0.37
	3	0.00	0.00	0.12	0.13	0.43	0.62
	6	0.00	0.00	0.06	0.22	0.91	0.90
22	1	0.00	0.00	0.24	0.31	0.53	0.51
	3	0.00	0.00	0.22	0.26	0.66	0.73
	6	0.00	0.00	0.24	0.35	0.96	0.88
24	1	0.00	0.00	0.02	0.10	0.42	0.48
	3	0.00	0.05	0.23	0.42	0.93	0.82
	6	0.00	0.02	0.48	0.64	0.95	1.00
26	1	0.00	0.00	0.00	0.00	0.67	0.64
	3	0.00	0.00	0.44	0.18	1.00	0.91
	6	0.00	0.00	0.60	0.56	1.00	1.00

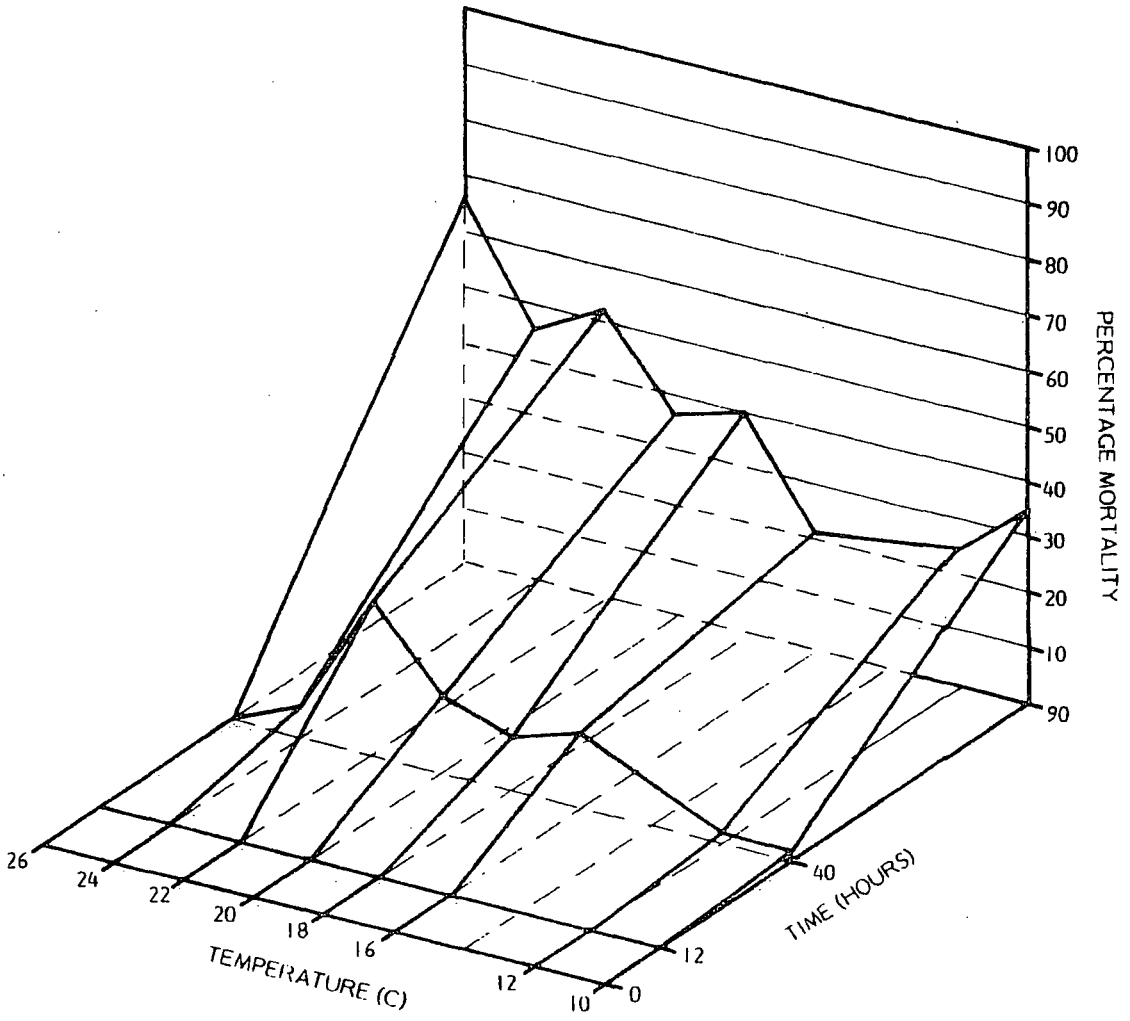


FIGURE 3.6-6

PERCENTAGE MORTALITY VERSUS TEMPERATURE AND TIME FOR 1 x 10^{-6}M GABA TREATMENTS: RED ABALONE LARVAL SETTLEMENT

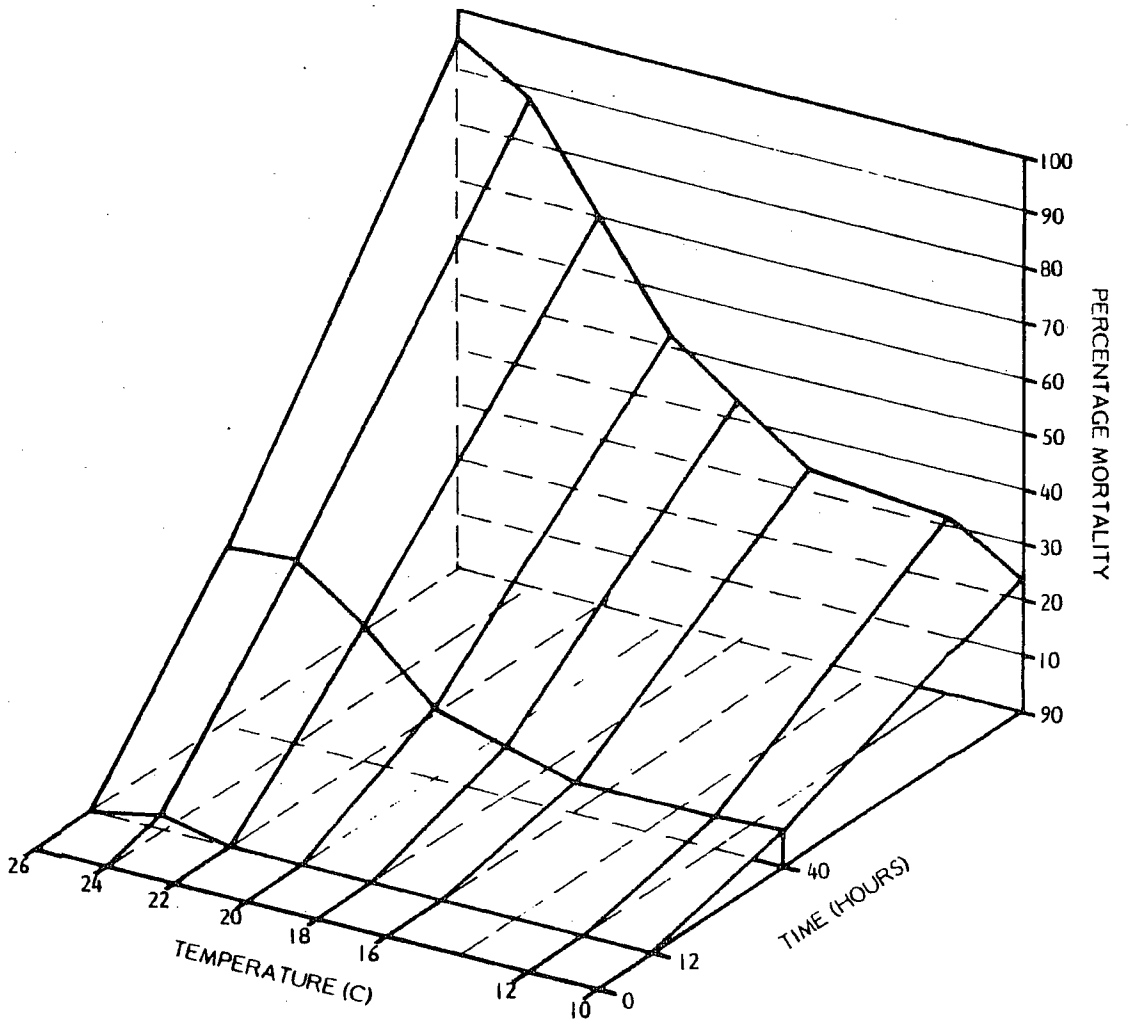


FIGURE 3.6-7

PERCENTAGE MORTALITY VERSUS TEMPERATURE AND TIME FOR 3×10^{-6}M GABA TREATMENTS: RED ABALONE LARVAL SETTLEMENT

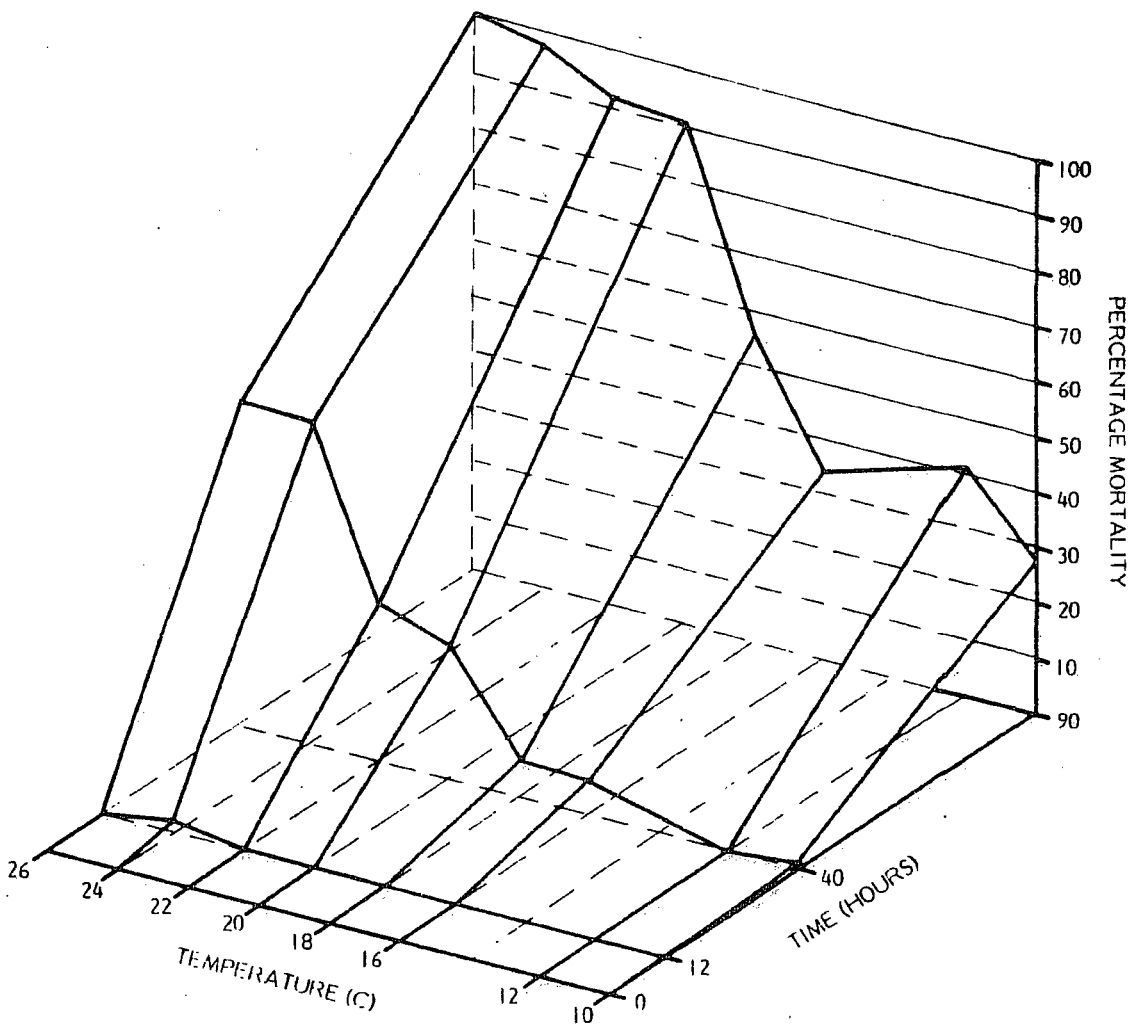


FIGURE 3.6-8

PERCENTAGE MORTALITY VERSUS TEMPERATURE AND TIME FOR 6 x 10^{-6}M GABA TREATMENTS: RED ABALONE LARVAL SETTLEMENT

TABLE 3.6-10

RESULTS OF MULTIPLE REGRESSION OF PERCENTAGE MORTALITY ON TEMPERATURE AND TIME: RED ABALONE LARVAL SETTLEMENT

Proportion mortality was transformed as follows:

$$\arcsin\sqrt{\text{proportion.}}$$

GABA concentration ($\times 10^{-6}$M)	Regression Equation*	R^2
1	$y = -15.4 + 0.5X_1 + 0.5X_2$	0.83
3	$y = -34.9 + 1.6X_1 + 0.6X_2$	0.84
6	$y = -53.1 + 2.4X_1 + 0.7X_2$	0.86

* X_1 = temperature (C), X_2 = time (h)

TABLE 3.6-11

ANALYSIS OF COVARIANCE OF PROPORTION MORTALITY ON TEMPERATURE, TIME AND GABA CONCENTRATION: RED ABALONE LARVAL SETTLEMENT

Proportion mortality was transformed as follows: $\arcsin \sqrt{\text{proportion}}$

Source	Sums of Squares	Degrees of Freedom	Mean Squares	F Value
Interaction Model[a]	12376.8	135	91.7	
Noninteraction Model[b]	16404.0	139	118.0	
Reduced Complete	4027.2	4	1006.8	$\frac{1006.8}{91.7} = 10.98$[c]

[a] Includes all interaction terms.

[b] Excludes all interaction terms.

[c] Significant.

TABLE 3.6-12

RESULTS OF TESTS OF EQUALITY OF SLOPE COEFFICIENTS FOR EACH TEMPERATURE TREATMENT OF PROPORTION MORTALITY VERSUS TIME: RED ABALONE LARVAL SETTLEMENT

Proportion mortality transformed as follows: arcsin $\sqrt{\text{proportion.}}$

GABA Concentration ($\times 10^{-6}$M)	Source	Degrees of Freedom	Sums of Squares	Mean Squares	F Value	Significant
1	Among b's	7	697.96	99.70	2.233	N.S.
	Error	32	1428.78	44.65		
3	Among b's	7	2148.74	306.96	6.638	*
	Error	32	1479.73	46.24		
6	Among b's	7	3260.96	465.85	8.894	*
	Error	32	1676.26	52.38		

* $p \leq 0.05$

TABLE 3.6-13

A POSTERIORI TEST FOR DIFFERENCES AMONG THE SLOPE COEFFICIENTS FOR EACH TEMPERATURE TREATMENT OF PROPORTION MORTALITY VERSUS TIME: RED ABALONE LARVAL SETTLEMENT

Proportion mortality transformed as follows: arcsin $\sqrt{\text{proportion}}$.

"X" = member of group.

GABA Concentration ($\times 10^{-6}$M)	Group	F (group)*	Temperature (C)							
			10	12	16	18	20	22	24	26
3	1	15.59	X	X	X	X	X			
	2	15.46			X	X	X	X		
	3	14.39					X	X	X	
	4	15.74							X	X
6	1	11.46	X	X	X	X				
	2	10.61					X	X	X	X

* F (group) $< F_{\alpha = 0.05}$

negating tests for individual treatments. If the factor combination means are plotted (FIGURE 3.6-9) all interaction occurs at temperatures below 18 C. At temperatures greater than 18 C, significant differences between treatment levels and the control would be expected.

Settlement

The mean proportions of settled larvae are plotted against time and temperature for GABA concentrations of 1×10^{-6}M in FIGURE 3.6-10, 3×10^{-6}M in FIGURE 3.6-11, and 6×10^{-6}M in FIGURE 3.6-12.

Multiple regression analysis was performed on the transformed settlement data at each concentration. The curvilinear response seen in FIGURES 3.6-10 to 3.6-12 is more distinct in FIGURES 3.6-13, 3.6-14, and 3.6-15. The "best fit" equation modeling this response contains higher order terms. Increasing mortality with time and differences between temperatures could explain these higher order terms. Analysis of covariance using dummy variables for the treatments yielded a significant test for interaction between treatments. Due to these difficulties, a test for differences between temperatures was performed using the mortality data.

Multiple regressions on the transformed mortality data at each concentration were performed and the results were presented in TABLE 3.6-10. Unlike the settlement data, mortality fits a first order linear model, lacking any interaction terms. Analysis of the two data sets, settlement and mortality, yielded a high correlation coefficient (-0.605). Due to the effect of mortality on settlement there was a high correlation and, in fact, mortality proved to be a predictor for settlement.

Evidence of new growth following settlement was recognized by the presence of peristomal shell. At 26 C no new shell was observed at any of the GABA concentrations. At 6×10^{-6}M GABA, no new shell was evident at 24 or 22 C. At the lower GABA concentrations, the most growth occurred in the 16 to 20 C

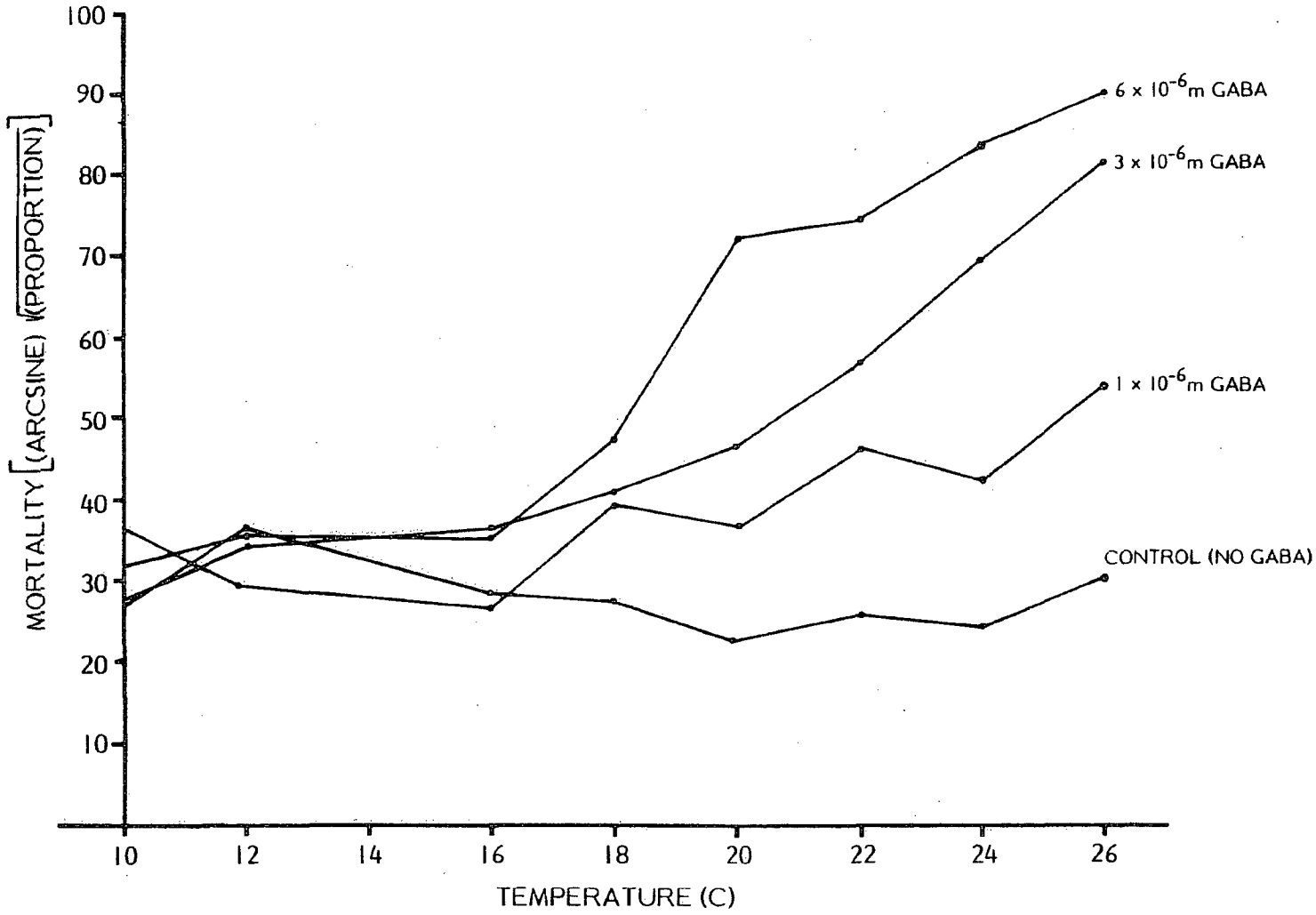


FIGURE 3.6-9

MORTALITY VERSUS TEMPERATURE AT 90 HOURS:
RED ABALONE LARVAL SETTLEMENT

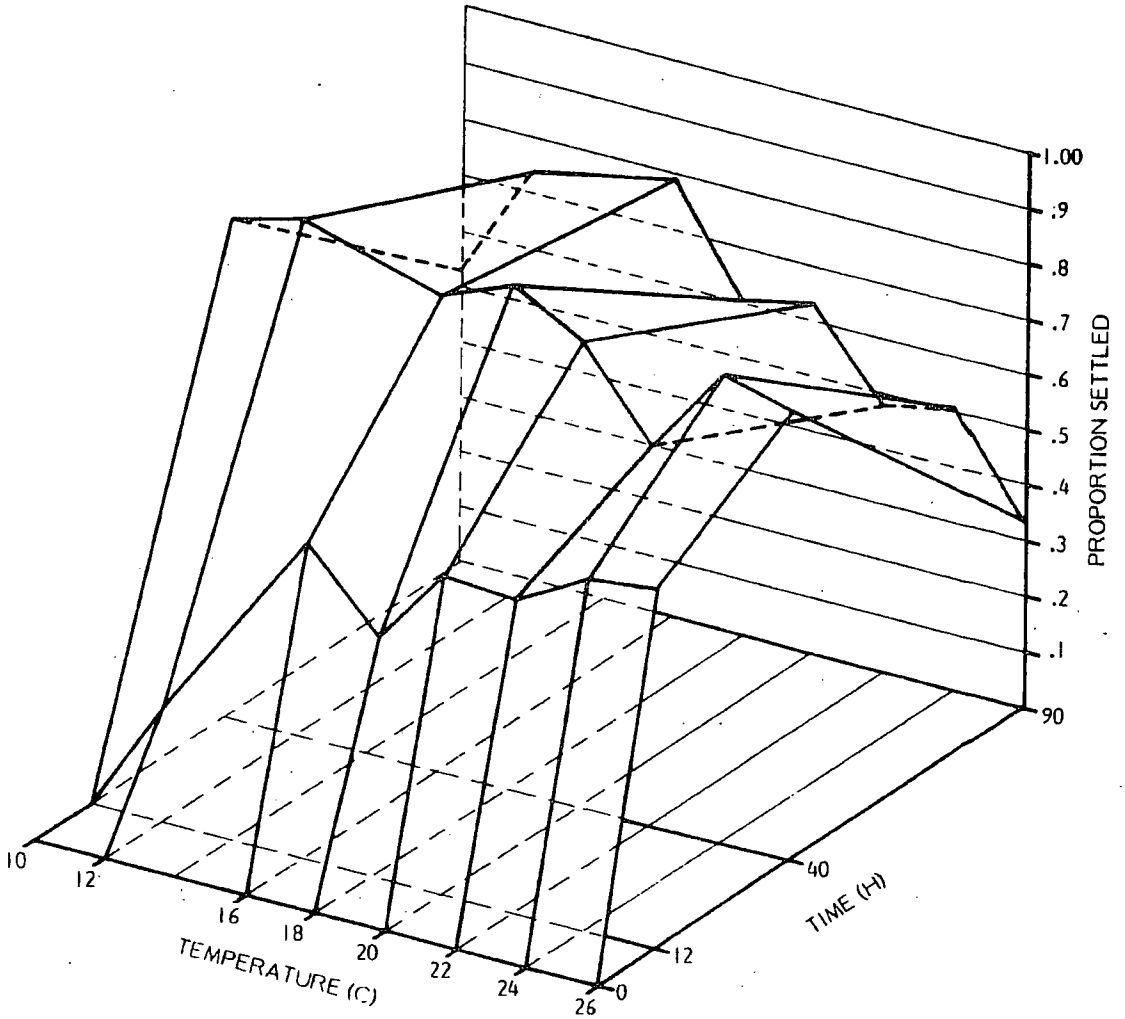


FIGURE 3.6-10

PROPORTION SETTLED VERSUS TEMPERATURE AND TIME IN 1×10^{-6} M GABA TREATMENTS: RED ABALONE LARVAL SETTLEMENT

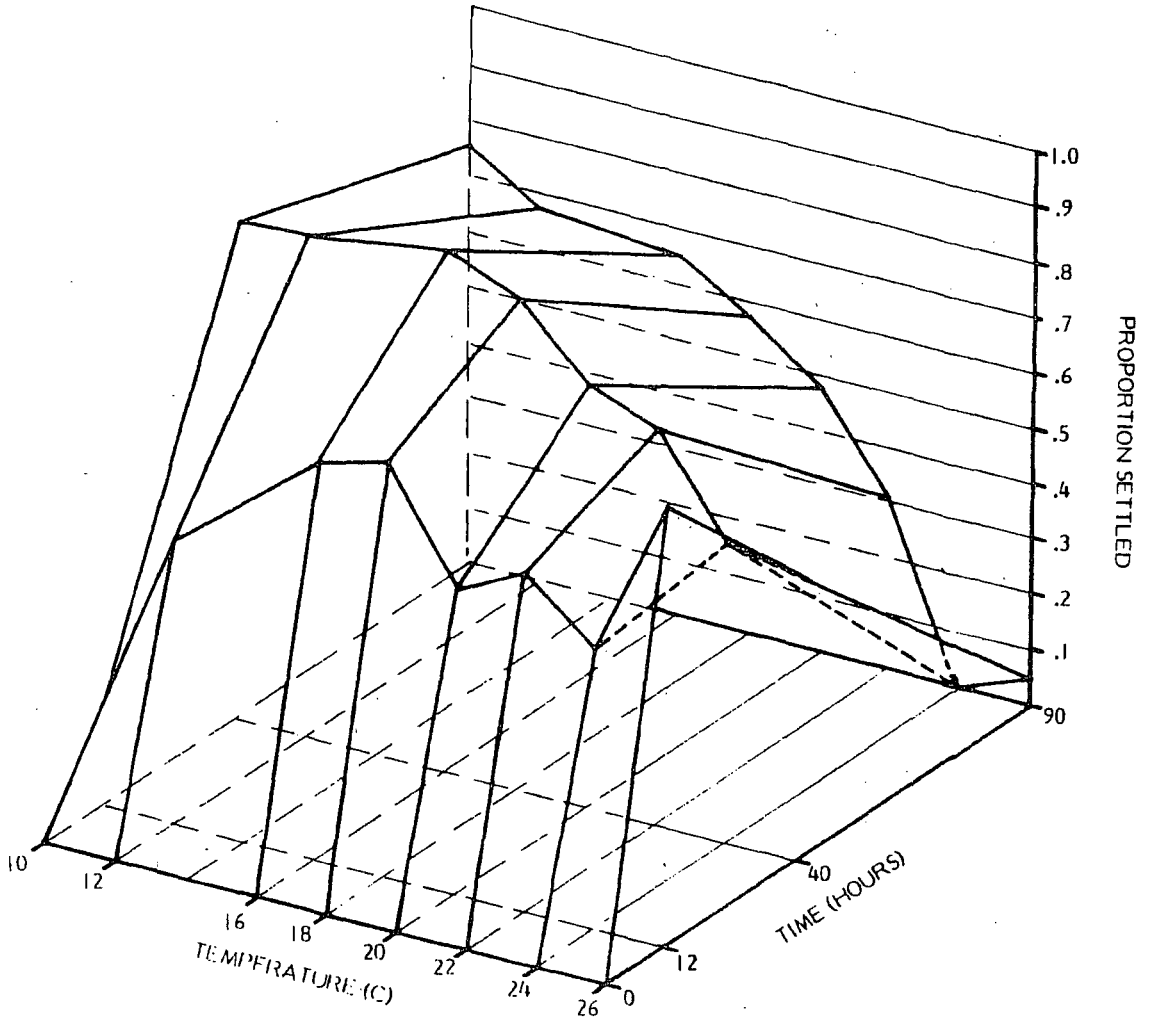


FIGURE 3.6-11

PROPORTION SETTLED VERSUS TEMPERATURE AND TIME IN 3 x 10^{-6}M GABA TREATMENTS: RED ABALONE LARVAL SETTLEMENT

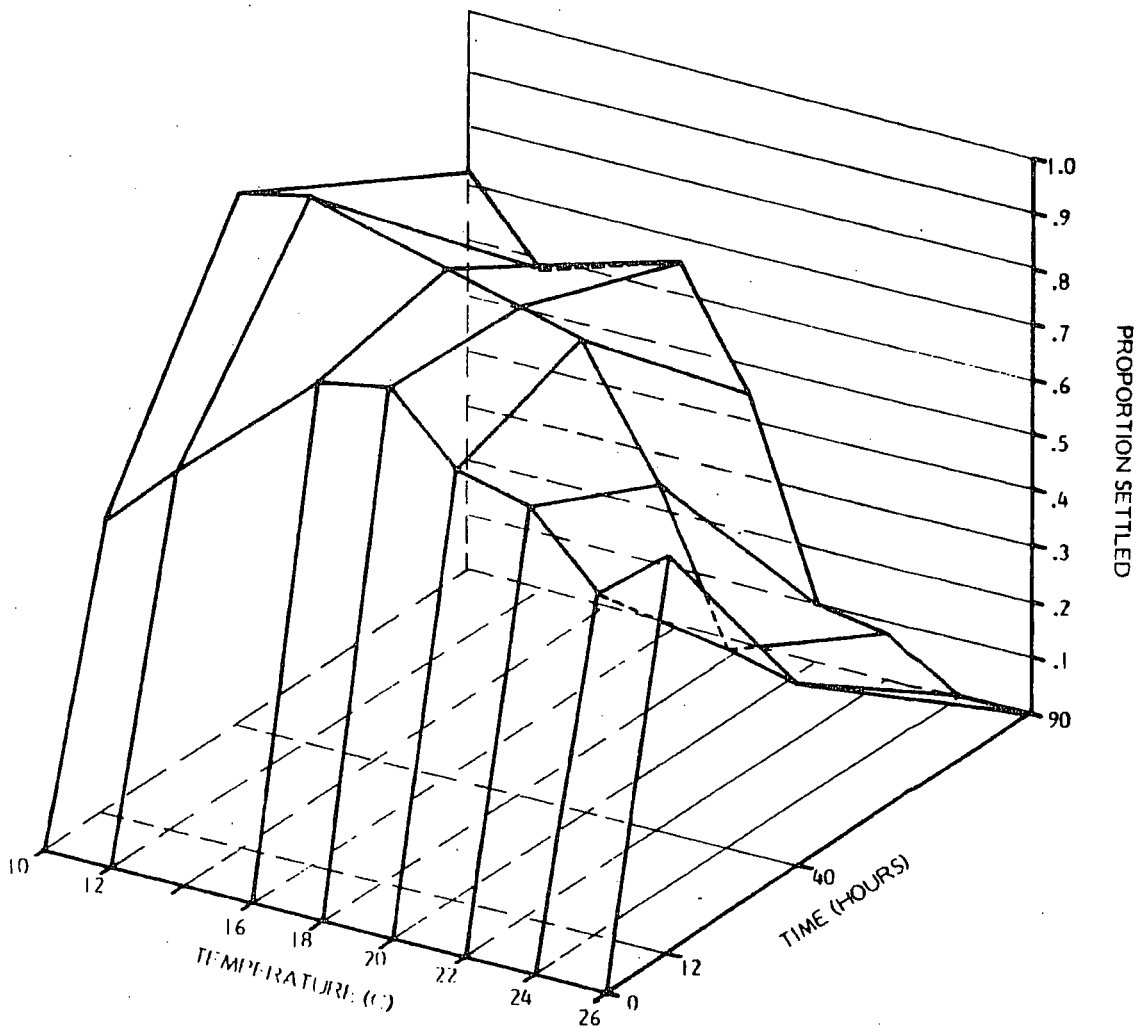


FIGURE 3.6-12

PROPORTION SETTLED VERSUS TEMPERATURE AND TIME IN 6 x 10^{-6}M GABA TREATMENTS: RED ABALONE LARVAL SETTLEMENT

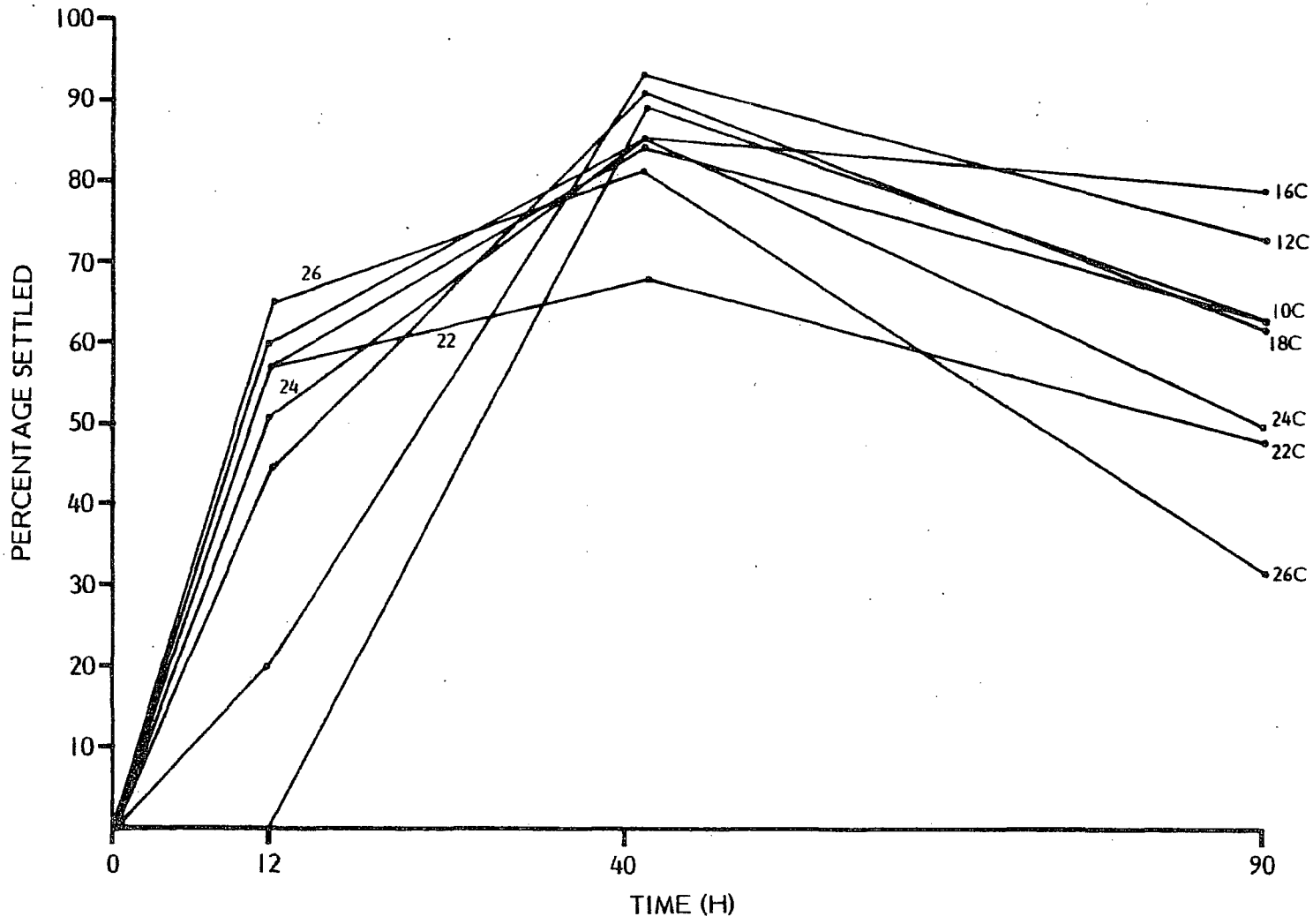


FIGURE 3.6-13

PERCENTAGE SETTLED VERSUS TIME
IN 3 x 10^{-6}M GABA TREATMENTS: RED ABALONE
LARVAL SETTLEMENT

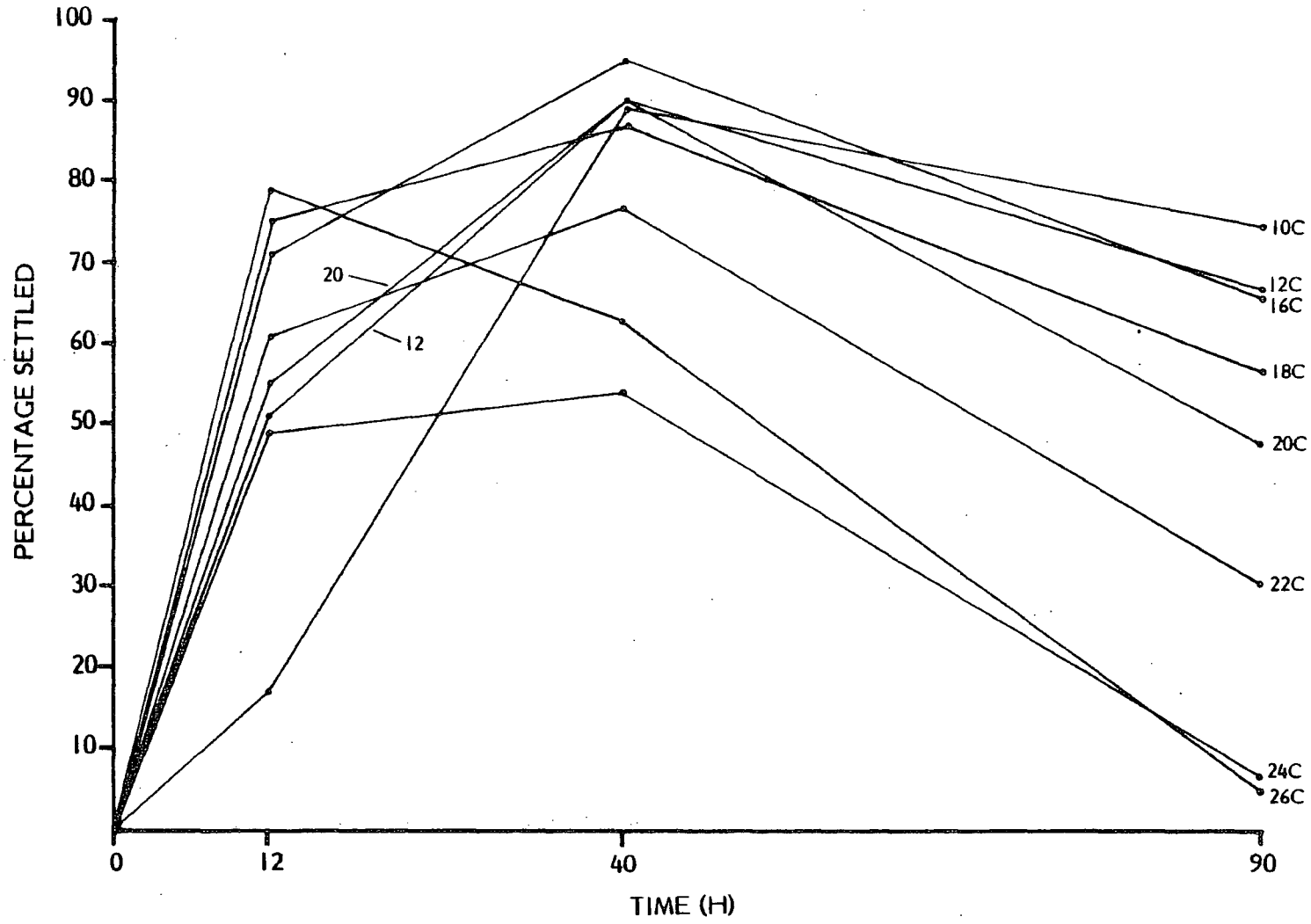


FIGURE 3.6-14

PERCENTAGE VERSUS TIME
IN 3 x 10^{-6}M GABA TREATMENTS: RED ABALONE
LARVAL SETTLEMENT

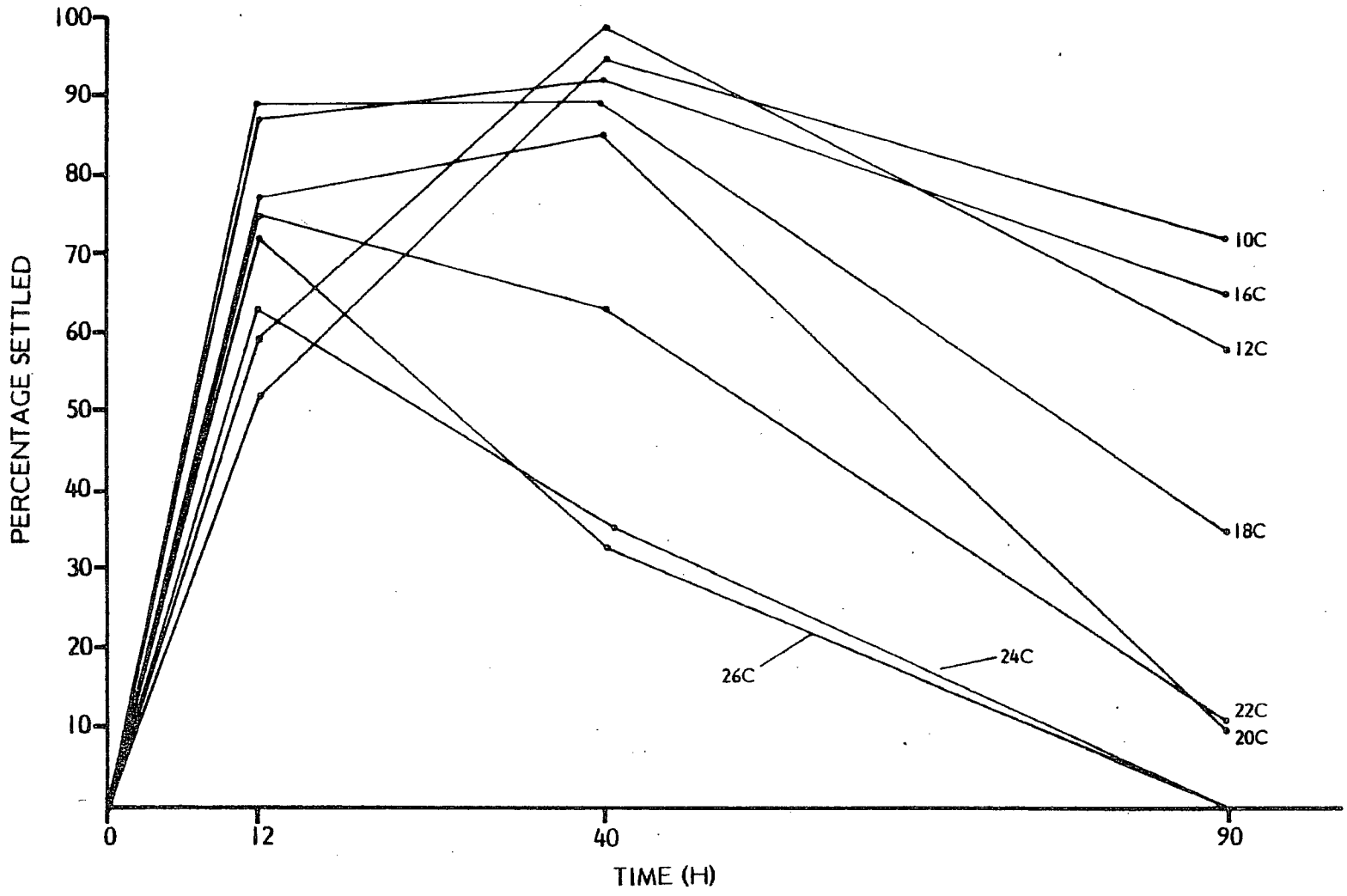


FIGURE 3.6-15

PERCENTAGE VERSUS TIME
IN 6 x 10^{-6}M GABA TREATMENTS: RED ABALONE
LARVAL SETTLEMENT

range. These results are consistent with reports by Leighton (1974) on juvenile growth in red abalone.

3.6.2.3 DISCUSSION

The effect of temperature on larval and juvenile development in red abalone was previously investigated by Leighton (1974). Time to settlement was measured on larvae held at the test temperature throughout larval life. Settlement occurred in four days at 18 C (64 F). The maximum temperature for survival increased with larval age and progressive stage of development. Leighton's findings are consistent with our results with the noteworthy exception that the results reported have shown the ability of veliger larvae to survive temperatures of 24 to 26 C (75 to 79 F).

Morse et al. (1979a, 1979b) found that GABA (γ-aminobutyric acid) induces larval red abalone to settle and begin metamorphosis. This inducer is obtained from crustose coralline and non-coralline algae. Morse et al. (1979b) determined that larvae held at 14-15 C (57-59 F) are competent for settlement 6 to 7 days after fertilization.

Sensitivity to temperature extremes is particularly acute at the two highest GABA concentrations. This is evidenced by the high level of mortality at temperatures exceeding 20 C (68 F). Even at the lowest GABA concentration thermal stress is evidenced by lack of any peristomal shell at temperatures above 22 C (72 F).

Actual concentrations of GABA available to larval abalone in nature are a matter of conjecture at present. Morse et al. (1979a) reported concentrations on the order of 10^{-2}M in their extractions from the corallines, Lithothamnium and Lithophyllum spp. At ambient temperature he reported the optimum concentration of GABA for settlement to be in the order of 10^{-6}M. From our results it is apparent that the optimal concentration for settlement is temperature dependent. The strong interaction between temperature and GABA concentration could be explained by the increased uptake of the substance at elevated

temperatures. Morse et al. (1979b) showed that at concentrations greater than 10^{-5}M, settlement was followed by a high level of mortality. Our studies reveal that at sub-lethal concentrations of GABA, mortality can occur at elevated temperatures. This effect decreases with decreasing GABA concentration, as is shown by the 1×10^{-6}M GABA treatment.

3.6.3 GROWTH

The purpose of the growth experiment on red abalone was to measure under laboratory conditions the growth rate of red abalone cultured in different combinations of temperature and algal diets. From this the optimal temperature and diet combination for growth can be obtained. In addition, the effects of the temperature and diet treatments on food consumption and mortality can be assessed. This information can provide an evaluation of the potential impact of changing ecological conditions which might be induced by the discharge of waste heat into Diablo Cove.

Growth rates of red abalone have been investigated by several authors (Cox 1962, Curtner 1917, Leighton 1968, Van Olst et al. 1978). Curtner (1917) used annual growth rings on red abalone shells to approximate age and derive growth rates. He calculated growth at approximately one-half inch per year. From the results of field observations and the recovery of tagged red abalone, Cox (1962) concluded that growth is not constant but varies considerably, even among individuals of the same size. He found that growth in abalone is directly dependent on the availablity of food.

The nutritional aspects of growth in red abalone have been studied by Leighton (1968). He found that the brown algae Macrocystis and Pterygophora were efficiently digested by the abalone. However, body growth on Macrocystis was far greater than on Pterygophora. Red algae were low in digestibility and provided little body growth. It was the conclusion of Van Olst et al. (1978) that growth rates in abalone changed in response to the fluctuation of nutrients in Macrocystis. The thermal ecology of the red abalone was first investigated by Leighton (1964). He found that adult red abalone had an upper lethal temperature

of 33 C for a one-hour exposure. Ebert (1974) confirmed this with a similar experiment and also found that acclimation to higher temperatures increased survival following thermal shock. This was demonstrated by PGandE (1979b) who found the 96-hour ET50 (i.e. the temperature at which 50 percent of the population survived) to vary in response to the initial acclimation temperature.

Red abalone acclimated at 12 and 15 C had ET50 values of 23.4 and 24.3 C respectively. Adams and Price (1974) found that significant mortality occurred among red abalone trochophore larvae exposed 10 minutes to 24 C water. Fertilization and cleavage stages were shown to be significantly affected above 20 C by PGandE (1980). In contrast to this, veliger larvae of red abalone are apparently unaffected by 24-26 C temperatures (PGandE 1980).

The optimum temperature for shell growth in red abalone is reported to be 15-18 C by McBeth (1972). Van Olst et al. (1978) found 19 C the optimum for shell growth; the optimal body growth temperature was 15 C. These adult optima are similar to the 18 C found by Leighton (1974) to be the optimal temperature for juvenile growth. Leighton (1968) found red abalone to feed throughout the range 7-26 C. Maximum feeding occured at 15-18 C.

3.6.3.1 METHODS

This experiment was run concurrently on four snail species: the black abalone (Haliotis cracherodii), the black turban snail (Tegula funebralis), the brown turban snail (Tegula brunnea), and the red abalone (Haliotis rufescens). Methods used were previously summarized in SECTION 3.5.2.1 for the black abalone. For a more detailed description of the methods used see APPENDIX B (Procedure 310) and protocol for experiment 10b (APPENDIX C). A brief outline of the critical aspects of the materials and methods as they relate to the red abalone is presented here. Approximately 350 hatchery reared red abalone were purchased from California Marine Associates, Cayucos, California. The sizes of individuals ranged from 30 to 80 mm in length. For a period of 30+ days the abalone were held in ambient seawater and fed to excess on Macrocystis. During this time they were carefully observed for any injury due to handling.

At the end of the initial observation period the animals were individually tagged and placed in 2x3x1 ft holding pans. These were covered with a fine mesh plastic screen, and placed in the appropriate 4x8 ft test tank. Flow rates in the test tanks were maintained at approximately 22.7 liters/min (6gpm). Test tank temperatures were slowly increased at the rate of 1 C/day until the appropriate test temperature was achieved. All tanks were monitored daily for mortalities. Each test tank was divided into three equal areas for the three diet treatments. Six test tanks were used -- one for each temperature treatment. Each diet compartment contained 16 red abalone. Each compartment also contained 30 Haliotis cracherodii, 30 Tegula funebralis, and 30 Tegula brunnea. The two species of Tegula were contained in habitats separate from the abalone. No effort was made to separate the species of abalone. Similar size distributions of red abalone were placed in each compartment. The total experimental population for the 18 treatment groups was 288 red abalone.

Test temperatures in each 4x8 tank were electronically controlled to within ±0.1 C of the specified test temperature throughout the course of the 90-day experiment. An unplanned power outage on day 6 caused larger temperature variations. The means, standard deviations, and ranges for the six temperature treatments were: 12.1+0.7 (9.9-13.7; ambient control), 12.0 + 0.3 (11.7-13.6), 14.8 + 1.3 (13.3-19.8), 17.9+0.4 (13.3-18.4), 21.0+1.2 (15.1-21.8), and 23.8+0.9 (14.2-24.2). The 90-day mean temperature for each treatment will be used for identification in this report. The three algal diets were composed of brown algae (Nereocystis luetkeana and Pterygophora californica), red algae (Iridaea flaccida and Botryoglossum farlowianum) and a mixed diet composed of roughly equal amounts of the four preceding algal species. The algal species were selected to represent the species of algae which commonly compose the algal drift found along the Diablo Cove shoreline. All three diets were fed twice a week to excess in each treatment group.

Twice during the experiment (day 17 and day 70), consumption was monitored over a three day period. This consisted of weighing the algae before and after the trial period. The difference was assumed to be the amount consumed. As black and red abalone were not separated, this figure represents the sum total of

both species' consumption. The wet caloric values of Paine and Vadas (1969) were used to estimate caloric intake. The dry caloric value of Cryptopleura violacea was used for that of Botryoglossum farlowianum. From the dry caloric value and percent water, the wet caloric value of Cryptopleura was calculated. Percentage water was calculated from information made available by California Department of Fish and Game at Diablo Canyon.

Maximum shell length and total weight were recorded at days 1, 49 and 91. Abalone were first anesthetized with MS-222 (Tricaine Methanesulfonate) at a concentration of 63.43 mg/liter until they could be easily removed. In addition, subsamples of body component weights in each test population were collected at the beginning and end of the experiment.

3.6.3.2 RESULTS

Preliminary results indicate that changes in the maximum shell length and body component weights are smaller among treatments in comparison to differences in total body weight. Therefore, in the final analysis which follows, only changes in body weight are used. Results for mortality and consumption are also presented.

Mortality

Mortality rates among red abalone on the 18 treatment levels was 6 percent overall (TABLE 3.6-14). A two-way analysis of variance (ANOVA) disclosed no significant differences in mortalities among diets ($p \leq 0.06$) or temperatures ($p = 0.31$). Except for two individuals, all mortalities occurred in the first 25 days of the experiment.

Consumption

Results of a two-way ANOVA (TABLE 3.6-15) reveal significant differences in consumption among diets and temperatures (both $p \leq 0.01$). The results of a Student-Newman-Keuls (SNK) multiple range test (TABLE 3.6-15) separates

TABLE 3.6-14

MORTALITY AMONG TREATMENTS:
RED ABALONE GROWTH

Mean Temperature (C)	Diet		
	Brown	Red	Mixed
12.0	0	0	0
12.1*	0	1	1
14.8	0	2	2
17.9	0	1	0
21.0	0	2	2
23.8	0	1	5

*Ambient Control

TABLE 3.6-15

TESTS OF SIGNIFICANCE OF CONSUMPTION DATA:
RED ABALONE GROWTH

Two-Way Analysis of Variance

Source	Degrees of Freedom	Sum of Squares	Mean Squares	F Value
Diet	2	155,094	77,547	101.4*
Temperature	5	25,985	5,197	6.8*
Error	10	7,647	764	

* Significant F-value at $\alpha = 0.05$

Student-Newman-Keuls Multiple Range Test*

Treatment Groups**

4	5	6	3	2	17	15	18	1	16	13	11	14	9	10	12	8	7

* Treatments are ordered according to the magnitude of their mean value. Treatments connected by underline are not significantly different.

** Treatment Group Codes

	Temp (C)					
	12.0	12.1	14.8	17.9	21.0	23.8
Brown Algal Diet	1	2	3	4	5	6
Red Algal Diet	7	8	9	10	11	12
Mixed Algal Diet	13	14	15	16	17	18

seven homogenous treatment groups. These subsets overlap considerably. The primary pattern of grouping pertains to diet -- brown algae was consumed the most, red algae the least. The mixed diet was intermediate in consumption (FIGURE 3.6-16). In general, consumption was greatest from 14.8-21.0 C. Above and below these temperatures consumption declined.

When the consumption data was transformed to caloric values, differences among diets became non-significant (p 0.10), while among temperatures differences were significant ($p<0.01$). A SNK multiple range test separated the temperatures into four homogenous subsets (TABLE 3.6-16). The amount of calories consumed was smaller at ambient temperature than at 12.0 C. More calories were consumed at 21.0 C than at any other temperature. At 14.8, 17.9 and 23.8 C caloric consumption was not significantly different (FIGURE 3.6-17).

Growth

As the abalone were measured after two approximately equal periods of growth, it is of interest to compare the results of these periods separately. A preliminary analysis of the homogeneity of group variances (Bartlett-Box, Cochrans and F-Max tests) indicated that group dispersions were unequal. To satisfy this important assumption of the ANOVA, the data was transformed to a logarithmic (base 10) scale.

Results for the two growth periods are different (TABLES 3.6-17 and 3.6-18). For the first growth period changes in body weight are significantly different among diets ($p< 0.01$), but not among temperatures (TABLE 3.6-17). A SNK multiple range test separates the diets into two subsets: brown algae, and red and mixed algae (TABLE 3.6-17). Thus, body growth is greater in red abalone consuming brown algae. There are no significant differences in body growth between the red and mixed algal diets. In the second growth period there are no significant differences among diets or temperatures (TABLE 3.6-18). These results are probably due to the highly variable response of weight in the treatment groups (FIGURE 3.6-18).

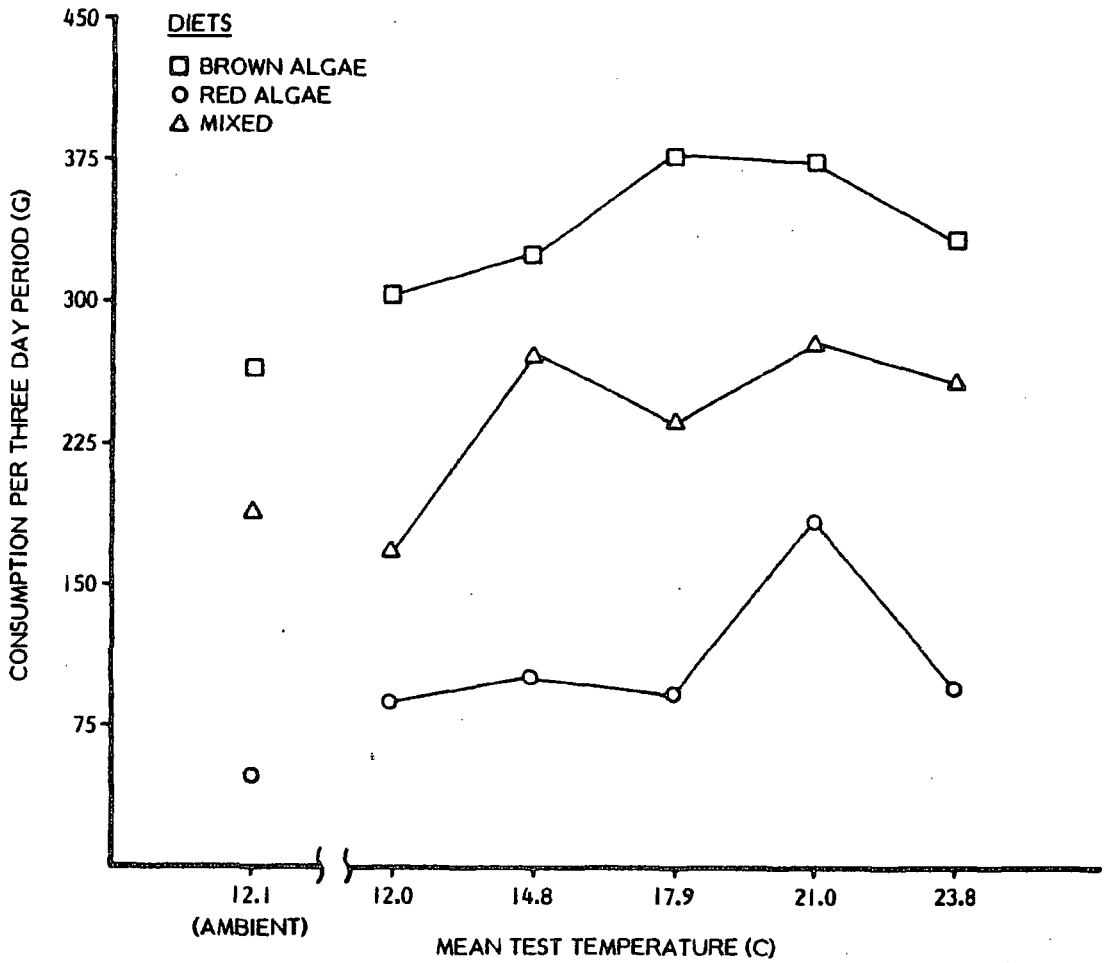


FIGURE 3.6-16

CONSUMPTION VERSUS TEMPERATURE:
RED ABALONE GROWTH

TABLE 3.6-16

TESTS OF SIGNIFICANCE OF CALORIC CONSUMPTION DATA:
RED ABALONE GROWTH

Two-Way Analysis of Variance

Source	Degrees of Freedom	Sum of Squares	Mean Squares	F Value
Diet	2	1,601	800.6	2.85
Temperature	5	10,153	2,030.7	7.23*
Error	10	2,806	280.65	

* Significant F-value at $\alpha = 0.05$

Student-Newman-Keuls Multiple Range Test*

Treatment (C)

12.1	12.0	14.8	23.8	17.9	21.0

* Treatments are ordered according to the magnitude of their mean value. Treatments connected by underline are not significantly different.

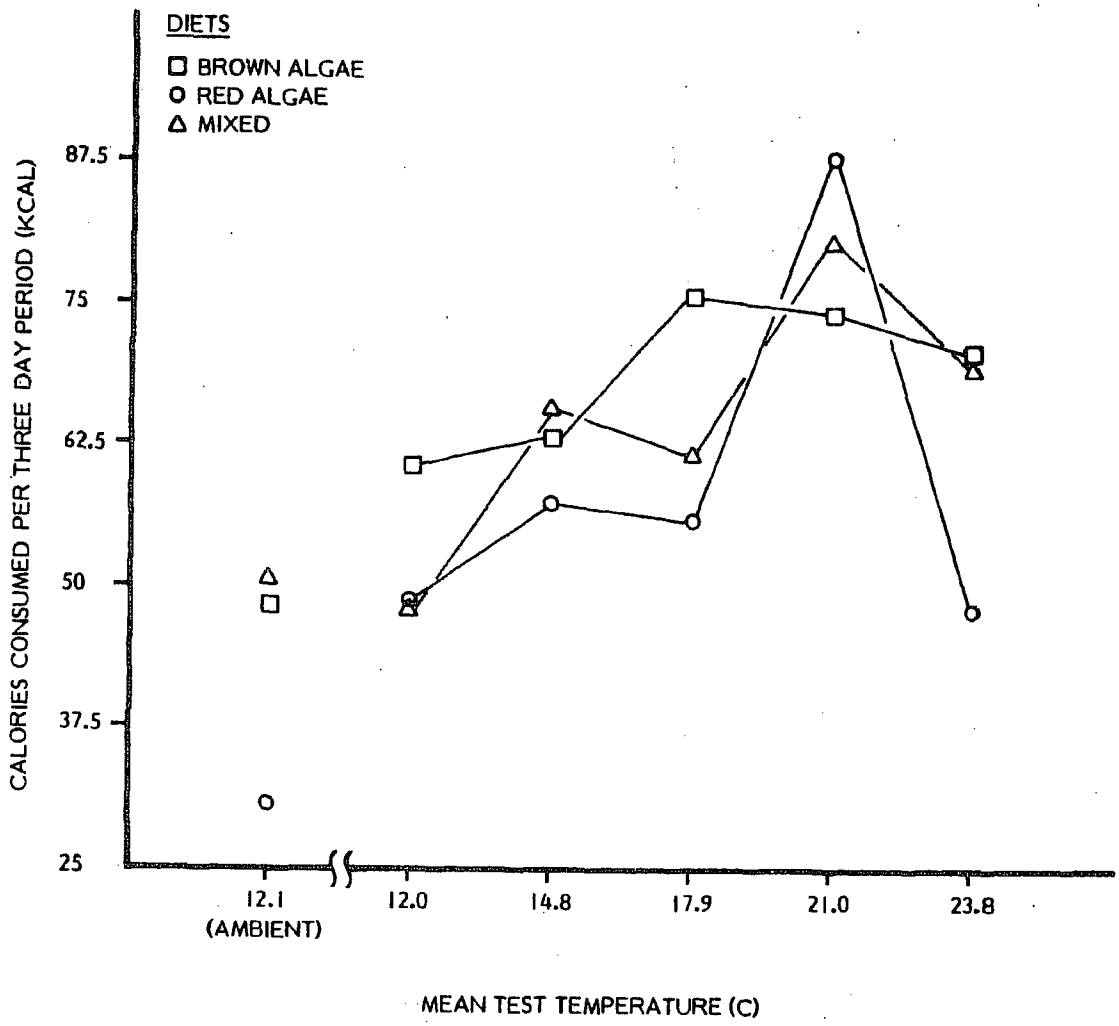


FIGURE 3.6-17

CALORIC CONSUMPTION VERSUS TEMPERATURE:
RED ABALONE GROWTH

TABLE 3.6-17

TESTS OF SIGNIFICANCE OF GROWTH FOR DAYS 1-49:
RED ABALONE GROWTH

Two-Way Analysis of Variance

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Significance of F
Main Effects	7	2.296	0.328	2.324	0.026
Temperature	5	0.586	0.117	0.830	0.529
Diet	2	1.730	0.865	6.127	0.003*
2-Way Interactions	10	2.234	0.223	1.583	0.112
Temperature and Diet	10	2.234	0.223	1.583	0.112
Explained	17	4.531	0.267	1.888	0.019
Residual	261	36.844	0.141		
Total	278	41.375	0.149		

* Significant F-value at $\alpha = 0.05$

Student-Newman-Keuls Multiple Range Test*

Algal Diet

Brown Mix Red

* Treatments are ordered according to the magnitude of their mean value. Treatments connected by underline are not significantly different.

TABLE 3.6-18

TESTS OF SIGNIFICANCE OF GROWTH FOR DAYS 49-91:
RED ABALONE GROWTH

Two-Way Analysis of Variance

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Significance of F
Main Effects	7	3.152	0.450	2.358	0.024
Temperature	5	1.987	0.397	2.081	0.068
Diet	2	1.118	0.559	2.927	0.055
2-Way Interactions	10	1.229	0.123	0.643	0.776
Temperature and Diet	10	1.229	0.123	0.643	0.776
Explained	17	4.381	0.258	1.349	0.162
Residual	261	49.840	0.191		
Total	278	54.220	0.195		

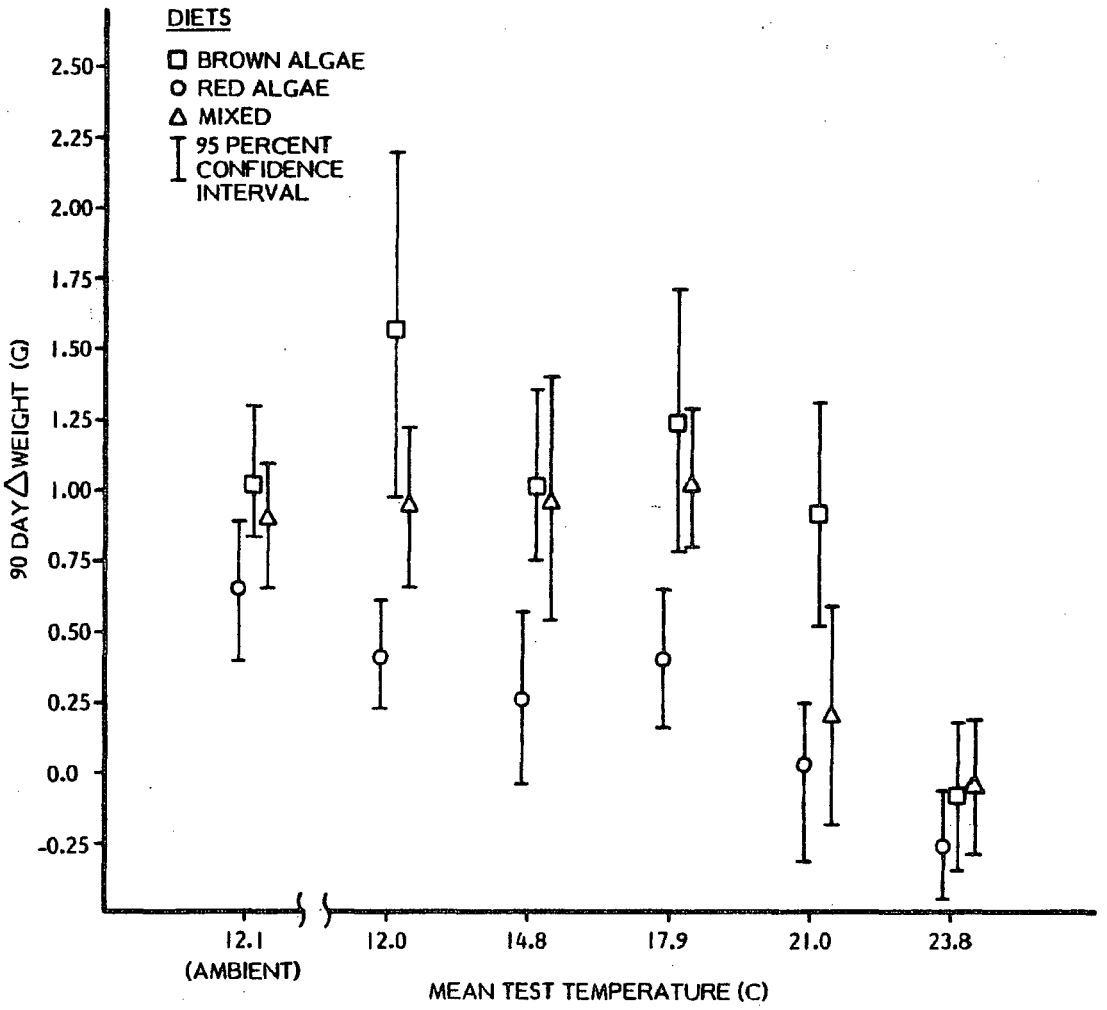


FIGURE 3.6-18

90 DAY GROWTH VERSUS TEMPERATURE:
RED ABALONE GROWTH

A two-way ANOVA of 90-day growth in the red abalone (TABLE 3.6-19) displays significant differences among diets and temperatures. An SNK multiple range test separates 8 homogeneous subsets (TABLE 3.6-19). These subsets display differences primarily due to diet, and secondarily due to temperature. Growth is greatest on the brown and mixed algal diets at 12.0-17.9 C, decreasing on the red algal diet and at temperatures greater than 17.9 C (FIGURE 3.6-18).

To test whether different size distributions in the treatments were influencing the results, an analysis of covariance was performed using initial weight as the covariate. The results are presented in TABLES 3.6-20 and 3.6-21 for the two growth periods. For both analyses the covariates are significant, but they do not change the significance of the main factors or interactions. From this it can be inferred that changes in body weight are directly related to the initial body weight. Due to similar size distributions in the treatments, however, adjustment of body weight growth relative to initial size does not change the overall results.

3.6.3.3 DISCUSSION

Due to the low rate of mortality it is not suprising that no significant differences exist in mortalities among treatments. This is consistent with the findings of Van Olst et al. (1978) who found that neither food type nor 13-19 C (55.4-66.2 F) temperatures had a significant effect on survival. Mortality in this experiment at the highest temperature (12 percent at 23.8 C (74.8 F)) was lower than that predicted by Ebert (1974; 20 percent at 23 C (73.4 F)) and PGandE (1979a; 50 percent at 23.4 C (74.1 F) in 12 C (53.6 F) acclimated animals). It is likely that the slow temperature increase (1 C/day) (33.8 F/day) and the presence of food helped to increase the red abalones thermal tolerance. The relationship between consumption and caloric consumption suggests that red abalone are regulating their caloric intake. In general, red algae have a higher caloric value than brown algae (Paine and Vadas 1969). If one assumes that caloric assimilation is approximately equal on all diets (as suggested in Olsen 1971), then the fact that there is no significant differences among diets in terms of calories supports this hypothesis. Studies on the green abalone (Haliotis fulgens) suggests that caloric intake is regulated to a certain degree (Olsen 1971). Thus, the apparent

TABLE 3.6-19

TWO-WAY ANALYSIS OF VARIANCE OF GROWTH FOR DAYS 0-91: RED ABALONE GROWTH

Two-Way Analysis of Variance					
Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Significance of F
Main Effects	7	3.579	0.511	25.914	0.000*
Temperature	5	2.594	0.519	26.295	0.000*
Diet	2	0.987	0.494	25.015	0.000*
2-Way Interactions	10	0.282	0.028	1.429	0.168
Temperature and Diet	10	0.282	0.028	1.429	0.168
Explained	17	3.861	0.227	11.511	0.000*
Residual	261	5.150	0.020		
Total	278	9.011	0.032		

* Significant F-value at $\alpha = 0.05$

Student-Newman-Keuls Multiple Range Test[1]

Experimental Group[2]

1 4 2 16 3 13 15 14 5 8 7 10 9 17 18 11 6 12

1 Treatments are ordered according to the magnitude of their mean value. Treatments connected by underline are not significantly different.

2

	Treatment Group Codes (C)					
	12.0	12.1	14.8	17.9	21.0	23.8
Brown Algal diet	1	2	3	4	5	6
Red Algal diet	7	8	9	10	11	12
Mixed Algal diet	13	14	15	16	17	18

TABLE 3.6-20

TWO-WAY ANALYSIS OF VARIANCE OF GROWTH ADJUSTED BY INITIAL WEIGHT FOR DAYS 1-49: RED ABALONE GROWTH

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Significance of F
Covariates	1	3.426	3.426	26.646	0.000
WT1	1	3.426	3.426	26.646	0.000
Main Effects	7	2.361	0.337	2.623	0.012
Temperature	5	0.605	0.121	0.941	0.455
Diet	2	1.778	0.889	6.914	0.001*
2-Way Interactions	10	2.160	0.216	1.680	0.086
Temperature and Diet	10	2.160	0.216	1.680	0.086
Explained	18	7.946	0.441	3.434	0.000
Residual	260	33.429	0.129		
Total	278	41.375	0.149		

* Significant F-value at $\alpha = 0.05$

TABLE 3.6-21

TWO-WAY ANALYSIS OF VARIANCE OF GROWTH ADJUSTED BY INITIAL WEIGHT FOR DAYS 49-91: RED ABALONE GROWTH

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Significance of F
Covariates	1	1.226	1.226	6.553	0.011
WT2	1	1.226	1.226	6.553	0.011
Main Effects	7	3.093	0.442	2.363	0.023
Temperature	5	1.959	0.392	2.095	0.066
Diet	2	1.086	0.543	2.904	0.057
2-Way Interactions	10	1.276	0.128	0.682	0.741
Temperature and Diet	10	1.276	0.128	0.682	0.741
Explained	18	5.595	0.311	1.662	0.046
Residual	260	48.626	0.187		
Total	278	54.220	0.195		

preference of red abalone for brown algae may be due to the lower caloric value of these algae which requires a larger dietary intake.

As temperature increases so does the metabolic rate of the exposed organism (Prosser 1973). The increase in consumption and caloric intake with temperature (FIGURES 3.6-16 and 3.6-17) suggests that the abalone are trying to maintain a constant energy balance. Above a certain temperature this caloric regulation may be adversely affected due to changes in the animals physiology. That caloric intake at 23.8 C (74.8 F) is less than that at 21.0 C (69.8 F) suggests that 21.0 C (69.8 F) may be a critical temperature for the red abalone.

In this experiment optimum body growth in the red abalone occurred while consuming brown algae at 12.0-17.9 C (53.6-64.2 F) (FIGURE 3.6-18). The annual average temperature in Diablo Cove is within this range. Van Olst et al. (1978) found 15 C (59 F) to be the optimal body growth temperature, while McBeth (1972) found maximum growth to occur at 15-18 C (59-64.4 F). These temperatures are within the range found in this experiment. It is likely that growth optima are different for abalone obtained from different geographical areas due to their unique genetic origin and acclimation temperatures. That the effects of diet on red abalone growth are more pronounced than the effects of temperature is significant. Studies in Australia (Shepherd 1973) and New Zealand (Poore 1972) have shown that the composition of the algal flora has a major effect on abalone growth, diet and food preferences.

3.7 BULL KELP (NEREOCYSTIS LUETKEANA)

3.7.1 GAMETOPHYTE GERMINATION AND EARLY DEVELOPMENT

During periods in the early development of many organisms, environmental conditions may affect not only rates of development but also the mode of development when qualitative developmental changes occur. This process can often be visualized as a series of stages or steps through which a normally developing individual proceeds. Under conditions different from those to which the species ordinarily is exposed, progression through these stages may be blocked at any stage.

The life history of the bull kelp involves two distinct generations. The conspicuous phase, the sporophyte, is an annual. The sporophyte reproduces by the asexual formation of spores, which are localized in dark patches called sori on the blades of the plant. The spores germinate and develop into either male or female gametophytes. The gametophytes represent the microscopic phase of the life history and are not observable in the field. The successful sexual reproduction of the gametophytes is critical, as it is the only way that new sporophytes can arise.

The effects of temperature and light intensity on the rate of zoospore germination and initial growth in the bull kelp (Nereocystis luetkeana) were studied in this experiment (experiment code 9a). Because this species has an obligate life history, in which the conspicuous sporophyte can only arise from sexual reproduction of the gametophytes, knowledge of the effects of temperature on gametophyte development and growth is crucial to an understanding of the effects of the discharge on this species. Because the gametophyte is microscopic and thus undetectable in the field, laboratory studies are the only way to determine these effects. The zoospores formed on the sporophytes from fall through spring, are released at various levels in the water column and are motile probably for a short time only. The spores settle on substrata of various types, metamorphose into nonmotile cells, germinate to form a germ tube and finally

form a vegetative multicellular filament. The subsequent stages of gametophyte growth and reproduction will be studied in a later experiment.

3.7.1.1 METHODS

A summary description of the laboratory methods employed in the investigations into spore germination and initial development of bull kelp is presented below. For a more detailed description of the laboratory methods see APPENDIX B (Procedure 309) and protocol see APPENDIX C (experiment 9a).

Pre-experimental

Prior to the experiment, it was necessary to determine the proper spore dilution to use so that the spores would not be crowded on the coverslips. Preliminary tests were also done on the uniform settling of spores in a large tray. Fertile material was collected in the field and brought back to the laboratory in an ice chest. The ripest sorus was cut away from the surrounding tissue, wiped several times in seawater and then dried on a damp towel in a 10C incubator for 15-30 min. The sorus was placed in 10C filtered seawater in a 2 liter beaker and returned to the 10C incubator without light. Spore release occurred within 30 min. The spent sorus was removed and the slime was allowed to settle out of the spore suspension (10-15 min). The suspension was carefully poured into a clean beaker and returned to the 10C incubator, where it was placed on a magnetic stirrer to keep the spores in suspension.

A small sample (2-4 ml) was taken from the well-stirred suspension and was mixed with 3-4 drops of 15 percent $MgSO_4$ in seawater in a small beaker. Four counts from the sample were counted on the compound microscope using a hemacytometer (TABLE 3.7-1). The average number of spores per mm^2 from the four counts was used to calculate the number of spores per ml of suspension (3.5×10^5). Several dilutions were made from the original suspension. Coverslips on glass rods were placed in the dilutions in large trays. The trays were kept in a 10C incubator without light. Coverslips were removed from both the middle and end of the trays after 24 and 48 hours. Spores were counted by locating 24

TABLE 3.7-1

HEMACYTOMETER COUNTS OF ZOOSPORES OF
NEREOCYSTIS FROM A SINGLE SAMPLE OF SPORE SUSPENSION

	Count			
	1	2	3	4
1	31	19	18	20
2	20	21	23	22
3	20	26	24	18
4	20	26	14	28
Total (= number per 0.25 mm^2)	91	92	79	88
x 4 = number per mm^2	364	368	316	352

microscope fields at 400x, using random number pairs and the mechanical stage of the microcsope. All spores in a field were counted and the sum of 25 fields was used to compute an average number of spores per field. A suspension containing approximately 7,000 zoospores per ml was selected for use, because it gave a spore set low enought to count in a reasonable period of time, yet gave a sufficient number of settled spores to yield consistent results between counts (TABLE 3.7-2). These results indicated that settlement of spores was uniform within a tray and did not increase from 24 to 48 hours.

Because most of the spores had germinated after 24 hours, it was necessary to determine the optimal time for spore settlement which yielded a high number of spores per field but with a low germination rate prior to sampling time. The results (TABLE 3.7-3) show an increase in the number of settled spores up to 6-12 hours. At 6 hours fewer spores had germinated than at 12 hours. Beyond 12 hours, germination increased as did settlement. A six-hour settling period was therefore chosen for this experiment.

Experimental

Fertile blades of bull kelp sporophytes were collected from an area west of Lion Rock on November 3, 1978. They were transported to the laboratory in a bucket and held in an ambient temperature raceway until experimental use. Zoospores were seeded onto coverslips before the beginning of the experiment using the above-described method.

The spores were allowed to settle in a dark 10C incubator. After 6 hours, one coverslip frame was placed in each of 22 culture dishes (80 x 100mm with blackened sides or totally blackened) containing 300 ml of half-strength PES culture medium at 10C. One coverslip frame was counted to determine the germination percentages attained during the settling period. The dishes were placed in water baths that had been brought to 9, 13, 17, 21 and 25C on the gradient table. Four dishes were placed in each water bath. Three of the dishes were exposed to light intensities of 44, 77 and 110 $\mu E/m^2/s$ and one dish was maintained as a dark control. Covers with openings for an air line and a

TABLE 3.7-2

ZOOSPORE COUNTS IN 25 FIELDS
FOR A SUSPENSION CONTAINING 7,000 CELLS PER ML

Time	24 hours						48 hours		
Tray position	middle			end			end		
Count number	1	2	3	1	2	3	1	2	3
Total number of zoospores	457	468	515	437	412	435	478	459	460
Mean no. of zoospores per field	18.3	18.7	20.6	17.5	16.5	17.4	19.1	18.4	18.4
Standard deviation	4.9	5.4	4.3	3.1	5.4	5.3	4.7	4.8	6.4

TABLE 3.7-3

TIME COURSE OF SETTLEMENT AND GERMINATION OF *NEREOCYSTIS* ZOOSPORES (7,000 CELLS/ML SUSPENSION)

	Settlement time (hours)						
	2	3	4	5	6	12	24
Total per 25 fields	139	147	155	186	327	339	628
Mean	5.6	5.9	6.2	7.4	13.1	13.6	25.1
Standard deviation	2.9	2.8	3.1	4.2	6.2	4.8	6.9
Germination	none	none	none	none	few	many	many

temperature probe were placed on all the dishes. For the dark controls, totally blackened dishes were used with black covers and black air lines. Neutral density screens were placed above the light dishes to obtain the required light intensities. A light dish (approximately 77 $\mu E/m^2/s$) and a dark dish were placed in an ambient temperature raceway (see TABLE 3.7-4).

Twenty four hours after the dishes were placed on the gradient table, and in the raceways, one coverslip was sampled from each dish. As each coverslip was removed it was placed in a numbered 10 ml beaker containing four percent formalin in seawater. The coverslips were observed using random number pairs as previously described and representative fields were photographed for some of them. This was repeated after 48 and 72 hours. If germination was greater than 95 percent after 48 hours, counts were not made at 72 hours. In addition, after 72 hours, the lengths of 25 gametophytes were measured (5 each in the 1st, 6th, 11th, 16th and 21st fields). These measurements were taken again after one week.

3.7.1.2 RESULTS

Two responses were measured in this experiment: 1) percentage germination based upon the formation of germ tubes from the settled zoospores, and 2) the increase in length of vegetative filaments produced from germinated zoospores. Germination counts were made at 0, 24, 48 and 72 hours and filament lengths were measured at 72 and 168 hours (7 days).

Germination

All germination values are means of 25 percentages obtained from one coverslip except the 0 hour controls which were made on two coverslips and the average of the two values was 2.1 percent. Due to initial problems in handling, one coverslip was lost from each of several treatments so that no data were obtained from those treatments at the 48 hour sampling. In TABLE 3.7-5 are presented all germination data obtained during the experiment. By hour 24 there is already a large difference between the germination percentage at 25C (about nine

TABLE 3.7-4

MATRIX OF TEMPERATURE AND LIGHT COMBINATIONS
TESTED IN EXPERIMENT 9a

Tested combinations are denoted by "x".

Light Intensity ($\mu E/m^2/s$)	Temperature (C)					
	9	13	14.8 (ambient)	17	21	25
(dark)	x	x	x	x	x	x
44	x	x		x	x	x
77	x	x	x	x	x	x
110	x	x		x	x	x

TABLE 3.7-5

MEAN PERCENTAGES OF GERMINATION OF
NEREOCYSTIS ZOOSPORES IN EXPERIMENT 9a

Sample time (hours)	Light Intensity ($\mu E/m^2/s$)	Temperature (C) 9	13	14.8 (ambient)	17	21	25
24	110	89.1	96.2	--	89.7	87.4	9.7
	77	89.5	95.5	95.9	96.3	87.9	9.5
	44	91.9	95.2	--	97.8	89.5	9.2
	(dark)	95.5	95.6	95.6	96.7	87.2	8.3
48	110	--	96.5	--	93.7	95.4	--
	77	97.5	97.2	95.8	97.3	93.8	5.2
	44	--	98.6	--	96.9	97.8	--
	(dark)	95.7	96.6	98.0	97.6	--	--
72	110	94.1	a	a	a	92.3	4.4
	77	96.4	a	--	a	95.4	4.8
	44	94.9	a	a	a	96.1	2.4
	(dark)	a	a	--	a	88.9	1.3

[a] No counts were taken after 95 percent germination was reached.

percent) and that at all other temperatures (greater than 85 percent). The 21C values are slightly lower in general than those in the 9, 13, 13.8 and 17C treatments. By 48 hours, the 21C percentages are comparable to those at the other temperatures (except 25C). By 72 hours, the 25C percentages have dropped, possibly due to mortality of previously germinated cells. In general, light intensity did not appear to have a marked effect, except that percentage germination frequently was lower at the highest light intensity. It is possible that high light intensities slow the rate of germination.

Growth

The few spores that germinated at 25C did not develop growing filaments. For the remaining treatments, filament lengths of 25 gametophytes from each coverslip were measured at days 3 and 7 (see TABLE 3.7-6). At day 3 the 21C filaments were much shorter than the filaments in all other treatments. The filaments appear to be shorter at the higher light intensities, which could be due to the lower germination rate mentioned above. At 7 days, the effects both of temperature and light intensity became more pronounced. The plants at 21C and those under dark conditions exhibited essentially no growth compared to the 3 day measurements. The plants at 9C had much shorter filaments than those at ambient, 13C and 17C. The plants were essentially as large at 77 $\mu E/m^2/s$ as they were at 100 $\mu E/m^2/s$, which indicates that light saturation of the plants was reached or approached in this experiment. The 7 day growth data for all treatments are plotted in a 3-dimensional graph (FIGURE 3.7-1) which illustrates the basic trends of the growth response.

3.7.1.3 DISCUSSION

Significant differences were detected in the effects of temperature and light on germination in contrast to growth. Whereas germination rates were essentially unaffected at 21C, growth was significantly lower at that temperature compared to the others tested. Light did not markedly affect germination but did have a strong affect on growth as is to be expected. The optimal temperature for initial growth appeared to be between 13C and 17C at 77-110 $\mu E/m^2/s$.

TABLE 3.7-6

MEAN LENGTHS IN μm (± STANDARD DEVIATION, N = 25)
OF FILAMENTS OF NEREOCYSTIS GAMETOPHYTES IN EXPERIMENT 9a

Sample Period (days)	Temperature (C)	Light Intensity ($\mu E/m^{-2}/s^{-1}$) (dark)	44	77	110
3	9	21.4(±2.6)	20.1(±3.2)	19.7(±3.1)	19.2(±3.3)
	13	20.5(±2.2)	19.7(±2.7)	19.8(±2.1)	19.1(±2.5)
	14.8 (ambient)	20.4(±2.6)	--	20.1(±3.5)	--
	17	20.0(±2.8)	20.8(±2.3)	18.7(±2.6)	18.8(±2.4)
	21	12.8(±1.7)	13.3(±1.6)	12.8(±2.0)	12.8(±2.0)
7	9	21.3(±3.1)	25.7(±4.1)	28.1(±4.7)	27.7(±6.4)
	13	20.1(±2.9)	45.1(±7.2)	49.9(±9.5)	46.7(±9.1)
	14.8 (ambient)	21.0(±2.7)	--	47.1(±11.8)	--
	17	19.0(±2.9)	43.0(±11.0)	51.8(±10.6)	52.2(±13.9)
	21	11.6(±1.7)	13.4(±1.9)	13.4(±2.1)	13.8(±1.2)

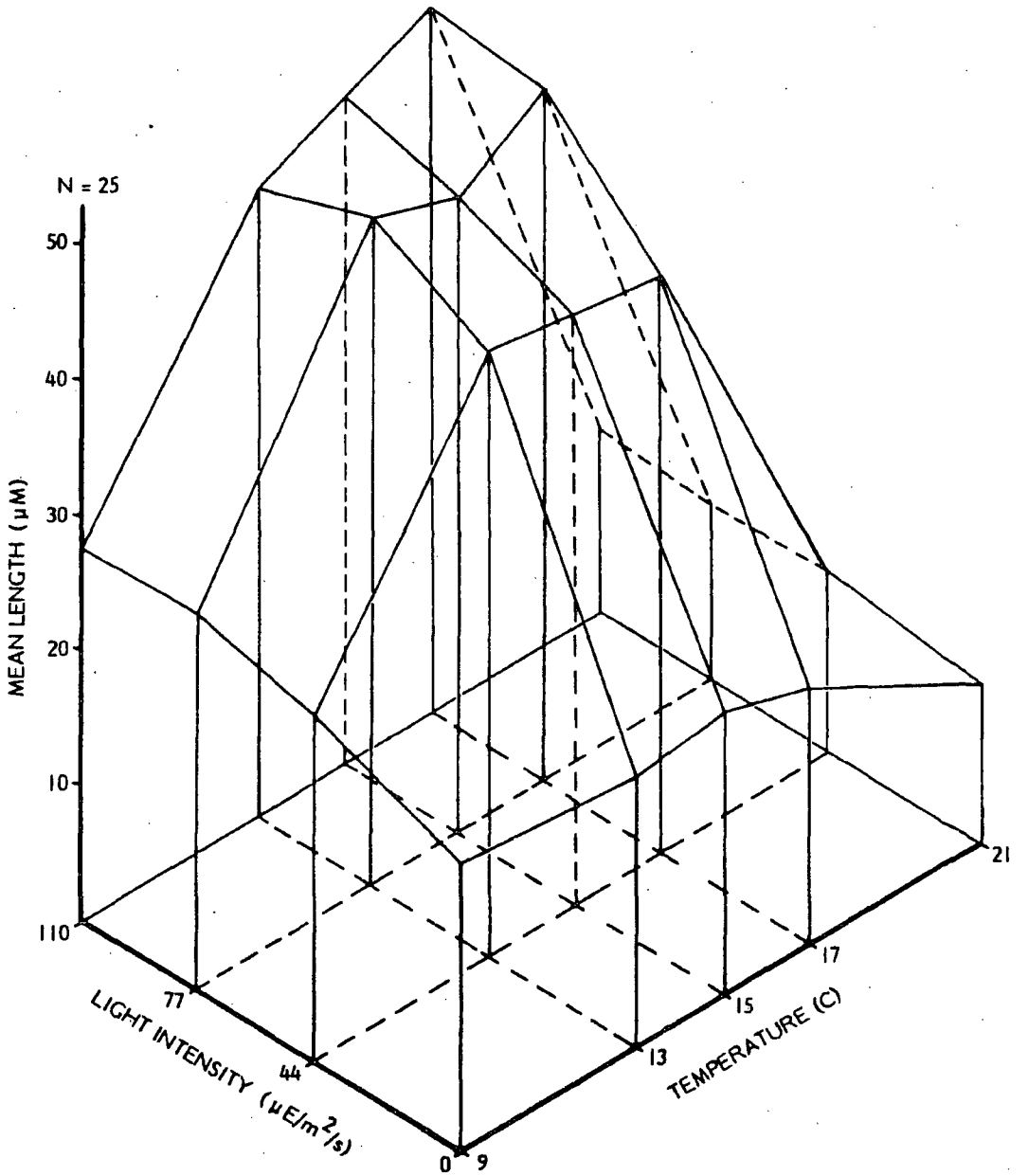


FIGURE 3.7-1

MEAN LENGTH OF NEREOCYSTIS LUETKEANA GAMETOPHYTE FILAMENTS AFTER 7 DAYS (EXPERIMENT 9A).

Fluctuating ambient temperatures did not appear to have a significant effect either on growth or on germination.

3.7.2 GAMETOPHYTE GROWTH AND FERTILITY

The results presented in the previous section revealed the effects of temperature and light intensity on spore germination and initial gametophyte growth. Successful initial growth, however, does not always ensure successful continued growth and fertility. The objective of this experiment was to determine the optimum temperature and light intensity for growth and fertility of established gametophytes and production of sporophytes. The purpose was to estimate the effects of the power plant thermal discharge on growth and reproduction of bull kelp gametophytes in Diablo Cove.

3.7.2.1 METHODS

Preliminary work with bull kelp leading to the development of procedures for the growth and fertility study is discussed below. The procedures are also summarized. More detailed information on procedures can be found in APPENDIX B (Procedure 318) and in APPENDIX C (protocol, experiment 18a).

Pre-experimental

Since this experiment involved the use of established gametophytes, it was necessary to determine the optimal culturing period that would yield plants at a stage still growing vegetatively and not showing fertility for at least a few days. In the previous section it was shown that one week old male and female gametophytes were not morphologically distinguishable from each other, so this age was used as a starting point. The week-old cultures were placed on the algal gradient table at various temperatures and were sampled after 2, 6 and 9 days on the table. These cultures were also used to develop procedures for staining the plants with a fluorescent dye. The dye acts as a marker to show the size of any plant at a certain point in time. When viewed under ultraviolet light in the microscope the dye in the plant cell walls will fluoresce, giving off a white glow.

Any new plant material added after staining will not have dye in the cell walls and will not glow. Using this technique, the amount of growth occurring during a known period can be measured.

The results of this experiment showed that the cultures were still growing vegetatively when they were 10 days old. The fluorescent dye technique worked very well to show this. Fertile males and females were present when cultures were 14 days old.

Experimental

Sori used for zoospore release were collected on 18 May 1979 south of Lion Rock from the surface by boat and transported to the laboratory where they were held at ambient temperatures until release was induced. The method of spore release and seeding of spores on coverslips was the same as in Procedure 309 (see APPENDIX B). To ensure good development of the gametophytes, they should not be crowded on the coverslips. For this reason a more dilute spore suspension was used (7,000 spores/ml for experiment 9a; 900 spores/ml for this experiment).

After the spores had settled on the coverslip assemblies, one frame was placed in each of 24 black culture dishes (80 x 100 mm). Each dish contained 300 ml of half-strength Provasoli's Enriched Seawater medium (PES). The dishes were covered and placed in a 13 C incubator with light intensity of approximately 32 $\mu E/m^2/s$. The cultures were grown without aeration for seven days with 16 hours of light and eight hours of dark per day.

After seven days, the coverslip frames were transferred to black culture dishes (80 x 100 mm) containing 300 ml of fresh culture medium at 13 C. One coverslip per dish was stained in fluorescent dye solution at 13 C and was returned to the coverslip frame at a specific location. The dishes were then placed in the appropriate water baths on the gradient table (four dishes per bath at 44, 77, 110 and 143 $\mu E/m^2/s$), covered and supplied with aeration. A temperature probe (YSI 423) connected to the Autodata Nine computer was inserted into each dish. The computer scanned the channels and provided a printout of the temperature

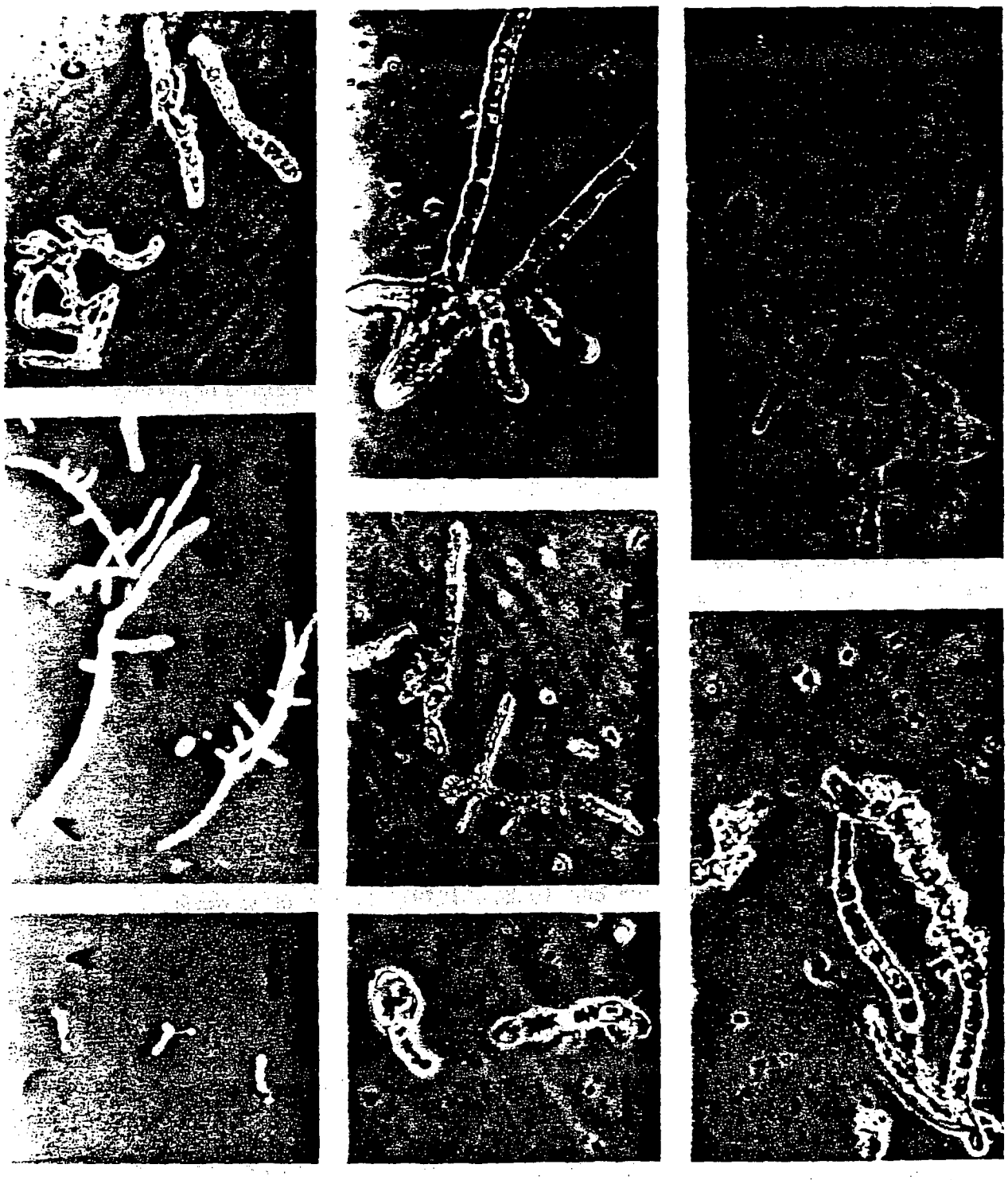
in each dish every hour. Gametophytes were measured to provide an average length at the beginning of the experiment.

The cultures were sampled after two days by removing the stained coverslip. Growth was assessed by measuring total filament length and the length of unstained filament for 30 randomly chosen gametophytes per coverslip. The percentage increase in length was computed from these figures. At each sampling period (up to six days on the gradient table) one coverslip was removed from each dish and was stained in fluorescent dye solution at the same temperature from which it was removed. These coverslips were used for the next set of growth measurements. Vegetative growth measurements were taken through day 6. By day 8, the gametophytes had developed extensive branching which made measurements difficult and time consuming. Therefore, measurements were discontinued at this time.

During the growth measurements, the gametophytes were checked for fertility. Random number pairs were used to locate plants for both the measurements and fertility rates. Fertility rates were determined by scoring fifty each of males and females as either fertile or non-fertile. If a female was fertile, the number of eggs and/or sporophytes per plant was counted. It was not possible to count the number of antheridia per fertile male.

3.7.2.2 RESULTS

The spores were set on 21 and 22 May. The experiment was initialized on 28 and 29 May and was terminated on 9 and 10 June 1979. In the presentation of results, the times given (e.g., "day 4") refer to elapsed exposure to treatment conditions. The age of the plants is equal to the time into treatment plus seven days. Photographs of the various stages seen during the experiment are presented in FIGURE 3.7-2. Insets A and B illustrate the value of the calco fluor white stain in detecting growth of the gametophyte filaments. In A (day 4 at 23 C) growth did not occur as shown by the uniform white fluorescence of the filaments. In B (day 10 at 16.7 C) growth is evident as unstained apical portions of filaments (arrows). Male (M) and female (F) plants were distinguished by



See Text for details. Scale bars = 40 μm in A, B. C. and E; = 20 μm in D, F, G and H

FIGURE 3.7-2

NEREOCYSTIS GAMETOPHYTE OPTIMUM TEMPERATURE FOR GROWTH AND FERTILITY: PHOTOGRAPHS ILLUSTRATING STAINING TECHNIQUE, MALE AND FEMALE MORPHOLOGY AND YOUNG SPOROPHYTE

marked differences in size and morphology, as shown in C (day 6, 12.9 C, 110 $\mu E/m^2/s$). Differences in the various treatments at day 10 are shown in D-G. Lack of fertility and an abnormal cell (arrow) are shown in D (19.8 C, 77 $\mu E/m^2/s$); a fertile male (M) with antheridia (arrow) and female (F) with an extruded egg (arrow) are shown in E (16.7 C, 143 $\mu E/m^2/s$); a female plant bearing several oogonia (arrows) is shown in F (17.8 C, 44 $\mu E/m^2/s$); and a fertile male with a cluster of antheridia (arrow) is shown in G (17.8 C, 44 $\mu E/m^2/s$). In H is shown a 19 d old female plant bearing a young sporophyte (arrow) (17.8 C, 143 $\mu E/m^2/s$). Details of the numerical data are presented in the following sections.

Temperature data

The means of the temperatures recorded for each dish throughout the entire experiment are shown in TABLE 3.7-7. In order to obtain high light intensities, high output fluorescent tubes were used on the gradient tables and the heat generated by these lights was enough to affect the temperature of the culture medium. The maximum and minimum values in TABLE 3.7-7 reflect the temperature fluctuations that occurred between day and night in each dish. For clarity, the mean value of the four mean temperatures for each light intensity is used in the following text in describing results: 8.6, 12.9, 16.7, 17.8, 19.8 and 23.0 C.

Vegetative growth

Length increase was measured directly using the fluorescent staining technique (see FIGURE 3.7-2A and B). The effects of temperature on percentage length increase are shown for day 2 in FIGURE 3.7-3, for day 4 in FIGURE 3.7-4 and for day 6 in FIGURE 3.7-5 (see also TABLE 3.7-8). Plants in the 23 C treatments were observed on day 6 to verify that no growth had occurred. In general, the same picture is evident at all three sample periods. The temperature for optimal growth is not well defined but lies in the range 12.9-17.8 C. A sharp drop in length increase occurs above 17.8 C and at 23.0 C no growth whatsoever was observed. One apparent anomaly occurred in the data from all

TABLE 3.7-7

STATISTICAL SUMMARY OF TEMPERATURE DATA FOR TREATMENTS: BULL KELP GAMETOPHYTE OPTIMUM TEMPERATURE FOR GROWTH AND FERTILITY

Channel	Light Intensity ($\mu E/m^2 sec$)	Temperature (C) Minimum	Maximum	Mean	Standard Deviation	No. of Records
113	110	14.6	18.0	16.7	0.97	n = 268
86	143	14.6	18.0	16.7	0.96	n = 269
128	77	14.6	18.0	16.7	0.88	n = 268
127	44	14.7	18.0	16.7	0.85	n = 268
126	110	11.7	14.5	13.0	0.63	n = 268
125	143	11.6	14.2	12.8	0.64	n = 268
124	77	11.6	14.1	12.8	0.61	n = 268
123	44	11.7	13.9	12.8	0.57	n = 268
122	110	8.3	9.3	8.7	0.22	n = 267
121	143	8.2	9.4	8.6	0.25	n = 267
120	77	8.2	9.3	8.6	0.22	n = 266
119	44	8.2	9.1	8.5	0.19	n = 267
115	110	21.9	23.7	23.0	0.59	n = 266
114	143	21.9	23.7	22.9	0.59	n = 270
118	77	22.0	23.7	23.0	0.59	n = 270
117	44	21.8	23.6	22.9	0.56	n = 270
88	110	18.2	20.7	19.7	0.81	n = 268
89	143	18.3	20.7	19.8	0.83	n = 270
90	77	18.3	20.7	19.8	0.68	n = 269
116	44	18.3	20.7	19.7	0.68	n = 270
85	110	16.4	18.7	17.8	0.56	n = 270
39	143	16.4	18.7	17.8	0.58	n = 268
40	77	16.5	18.7	17.8	0.75	n = 270
87	44	16.4	18.7	17.8	0.76	n = 270

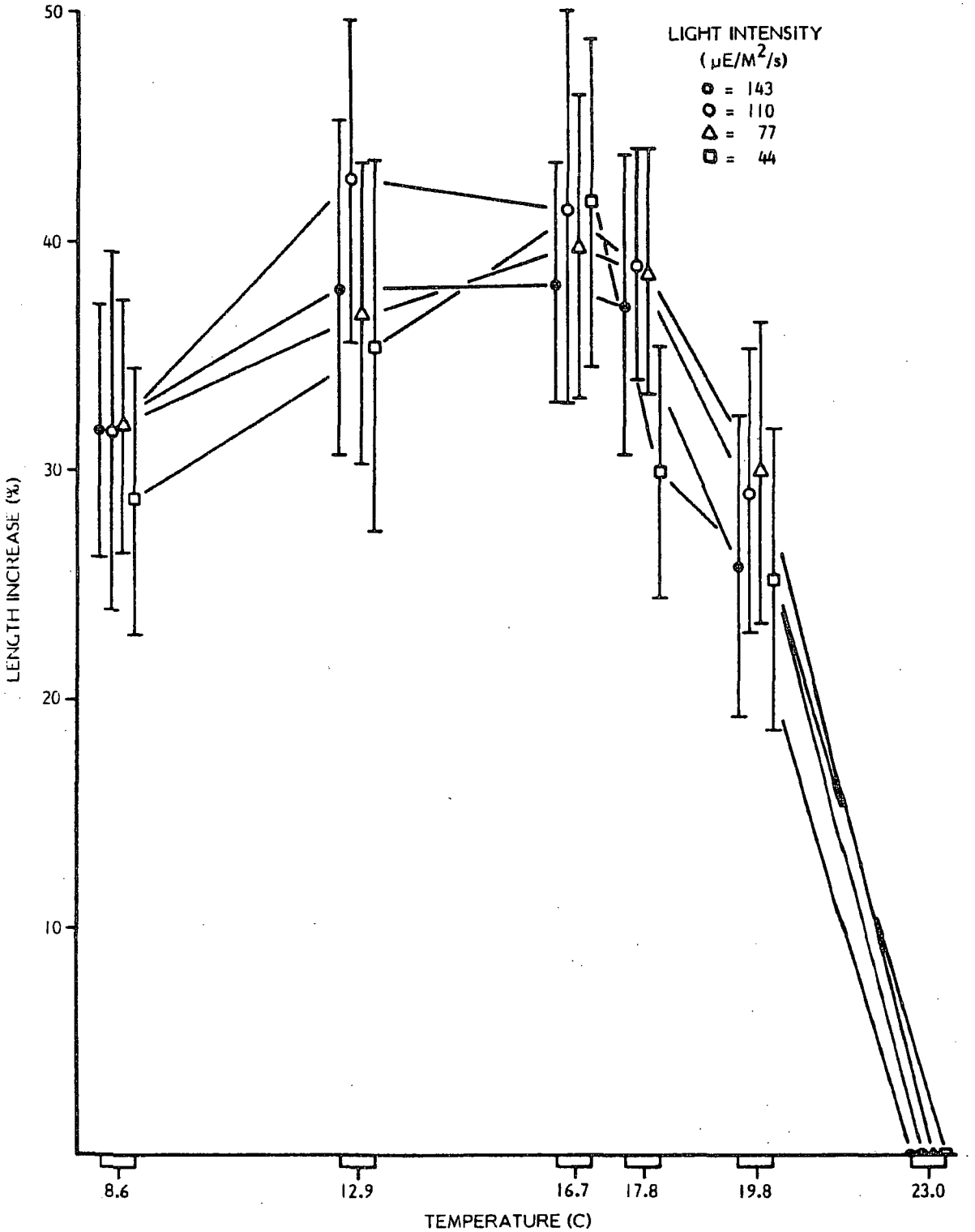


FIGURE 3.7-3

PERCENTAGE INCREASE IN FILAMENT LENGTH BY DAY 2 VERSUS TEMPERATURE AT FOUR LIGHT INTENSITIES: BULL KELP GAMETOPHYTE OPTIMUM TEMPERATURE FOR GROWTH AND FERTILITY

Standard deviations are shown on vertical lines. At 23 C, percentage length increase was zero at all light intensities.

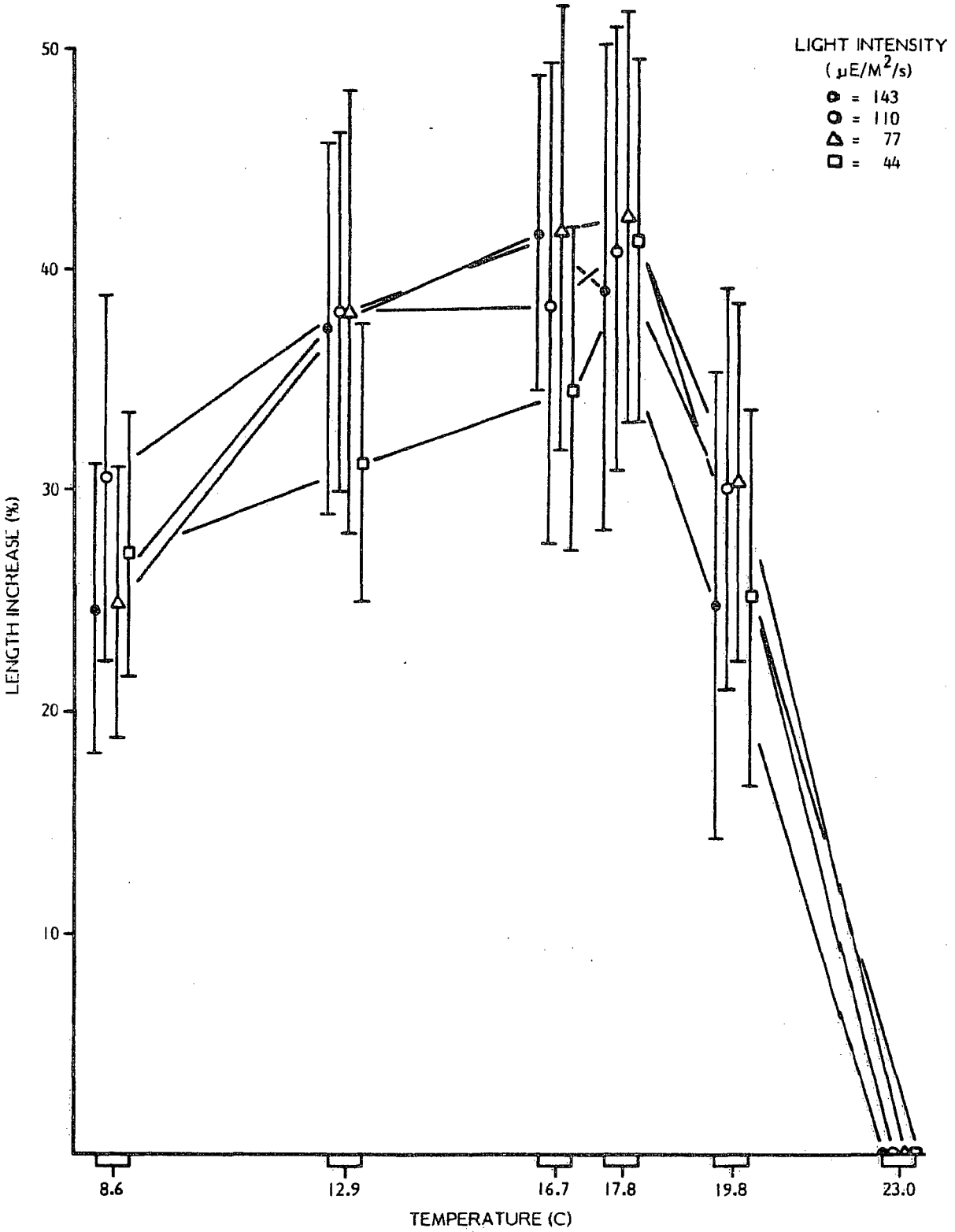


FIGURE 3.7-4

PERCENTAGE INCREASE IN FILAMENT LENGTH BY DAY 4 VERSUS TEMPERATURE AT FOUR LIGHT INTENSITIES: BULL KELP GAMETOPHYTE OPTIMUM TEMPERATURE FOR GROWTH AND FERTILITY

Standard deviations are shown on vertical lines. At 23 C, percentage length increase was zero at all light intensities.

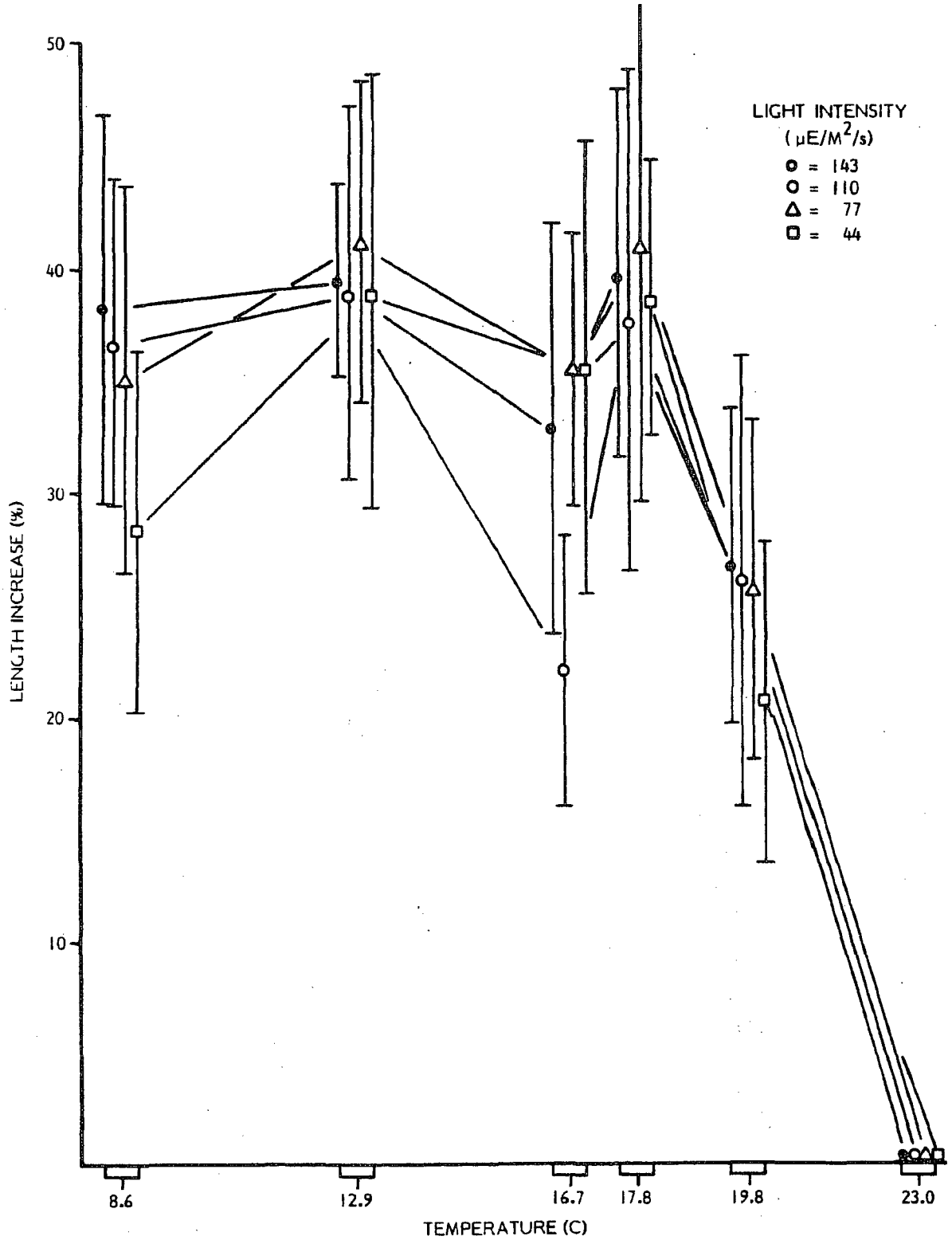


FIGURE 3.7-5

PERCENTAGE INCREASE IN FILAMENT LENGTH BY DAY 6 VERSUS TEMPERATURE AT FOUR LIGHT INTENSITIES: BULL KELP GAMETOPHYTE OPTIMUM TEMPERATURE FOR GROWTH AND FERTILITY

Standard deviations are shown on vertical lines. At 23 C, percentage length increase was zero at all light intensities.

TABLE 3.7-8

PERCENTAGE LENGTH INCREASE OF FILAMENTS:
BULL KELP GAMETOPHYTE OPTIMUM TEMPERATURE
FOR GROWTH AND FERTILITY

The means are given with standard deviations in parentheses.

Sample Time (days)	Light Intensity ($\mu E/m^2/s$)	Mean Temperature (C)					
		8.6	12.9	16.7	17.8	19.8	23.0
2	143	31.8 (5.5)	38.0 (7.2)	38.2 (5.2)	37.2 (6.6)	26.3 (6.7)	0
	110	31.7 (7.7)	42.8 (7.0)	41.4 (8.5)	39.0 (4.9)	29.0 (6.3)	0
N = 30	77	32.0 (5.5)	36.9 (6.6)	39.9 (6.6)	38.7 (5.8)	30.0 (6.5)	0
	44	28.8 (5.8)	35.4 (8.1)	41.8 (7.0)	30.0 (5.5)	25.9 (6.6)	0
4	143	24.6 (6.6)	37.3 (8.4)	41.5 (7.0)	39.0 (10.9)	25.2 (10.6)	0
	110	30.4 (8.3)	38.1 (8.1)	38.3 (10.8)	40.7 (9.9)	30.1 (9.1)	0
N = 30	77	24.8 (6.1)	38.0 (10.2)	41.7 (10.0)	42.3 (9.2)	30.4 (8.0)	0
	44	27.1 (5.6)	31.1 (6.3)	34.4 (7.4)	41.2 (8.1)	25.9 (8.5)	0
6	143	38.2 (8.5)	39.3 (4.3)	32.8 (9.1)	39.6 (8.0)	26.6 (7.3)	0
	110	36.5 (7.2)	38.7 (8.4)	22.1 (6.0)	37.5 (11.0)	26.0 (10.0)	0
N = 15	77	34.9 (8.5)	41.1 (6.9)	35.4 (5.9)	40.8 (10.8)	25.9 (7.6)	0
	44	28.2 (8.0)	38.8 (9.6)	35.5 (10.2)	38.5 (5.9)	21.7 (7.3)	0

of the 16.7 C treatments in that the increases appear to be depressed (FIGURE 3.7-4). It is assumed that these results are not caused by a temperature effect. It is possible that the growth rate decreased as the plants became reproductive but if this were true, a similar depression would have been expected in the 17.8 C treatments (see below). From these data, light intensity does not appear to have had a significant effect on growth rates.

Means of the total length measurements are given in TABLE 3.7-9 and three dimensional plots of the means are given in FIGURES 3.7-6 to 3.7-8 for days 2, 4 and 6, respectively. Total length measurements were not made at 23 C on day 6 because no growth had been observed. The effects of temperature become more pronounced with time, but the general picture is consistent in that the peak of total length generally occurs at 16.7 C. It also appears that the highest light intensity (143 $\mu E/m^2/s$) produced a greater total length than did the lower light intensities. As with the percentage increase data, a marked drop off in growth is evident above 17.8 C (note that measurements were not taken in the 23.0 C treatment on day 6). The general effects of temperature as shown in these results are in agreement with the results of a previous experiment (PGandE 1979a) in which week-old gametophytes exhibited peak growth at 17 C. Other kelp genera, such as Pterygophora, are also reported to have gametophyte growth optima at or near 17 C (see SECTION 3.8.2 of this report and LUnning and Neushul 1978).

Fertility

Illustrations of the various fertile stages are presented in FIGURE 3.7-2 E to G. No fertile plants were observed in the 19.8 and 23.0 C treatments during the experiment. Data for the remaining treatments are presented in TABLES 3.7-10 and 3.7-11. Plants were checked for fertility on days 2, 4 and 6 when no fertile plants were observed. Reproductive structures were first observed on day 10 at which time more than 90 percent of the male plants were fertile in all 17.8 C treatments and in the 143 and 100 $\mu E/m^2/s$ treatments at 16.7 C (FIGURE 3.7-9). About 30-75 percent of the female plants were also fertile at 17.8 C with considerably lower percentages or lack of fertility at lower temperatures

TABLE 3.7-9

MEAN TOTAL FILAMENT LENGTH:
BULL KELP GAMETOPHYTE OPTIMUM TEMPERATURE
FOR GROWTH AND FERTILITY

The means are given with standard deviations in parentheses.

Sample Time (days)	Light Intensity (μ E/m^2/s)	Mean Temperature (C)					
		8.6	12.9	16.7	17.8	19.8	23.0
2	143	57.7 (7.0)	65.5 (8.3)	70.7 (11.5)	54.6 (8.0)	50.9 (7.4)	33.3 (8.8)
	110	58.3 (8.5)	68.0 (10.8)	70.5 (10.0)	61.2 (11.3)	54.8 (9.5)	37.1 (8.0)
N = 30	77	60.9 (9.3)	61.9 (8:8)	63.1 (10.1)	65.5 (9.9)	58.0 (8.1)	33.3 (5.8)
	44	56.0 (7.6)	63.4 (8.6)	68.4 (8.8)	59.8 (9.9)	53.1 (5.8)	39.3 (7.2)
4	143	80.8 (12.5)	93.2 (18.8)	129.2 (41.8)	101.1 (25.1)	72.2 (16.4)	36.6 (7.2)
	110	67.0 (17.9)	94.6 (23.7)	98.3 (24.0)	98.8 (29.6)	77.3 (18.9)	37.0 (7.1)
N = 30	77	69.3 (13.4)	93.9 (14.3)	106.2 (25.1)	84.4 (23.7)	72.9 (15.4)	38.8 (6.6)
	44	74.4 (16.5)	91.1 (17.8)	98.7 (19.9)	94.7 (27.9)	75.8 (15.0)	43.1 (9.6)
6	143	113.7 (32.3)	144.6 (45.6)	175.5 (38.1)	114.3 (32.2)	89.7 (22.3)	
	110	114.0 (25.0)	148.3 (43.0)	143.1 (30.1)	142.4 (39.3)	83.9 (19.8)	
N = 15	77	88.0 (20.9)	134.1 (28.6)	155.5 (43.1)	150.7 (29.8)	88.6 (27.9)	
	44	105.3 (22.5)	128.2 (22.8)	151.9 (36.8)	134.9 (40.8)	79.7 (20.5)	

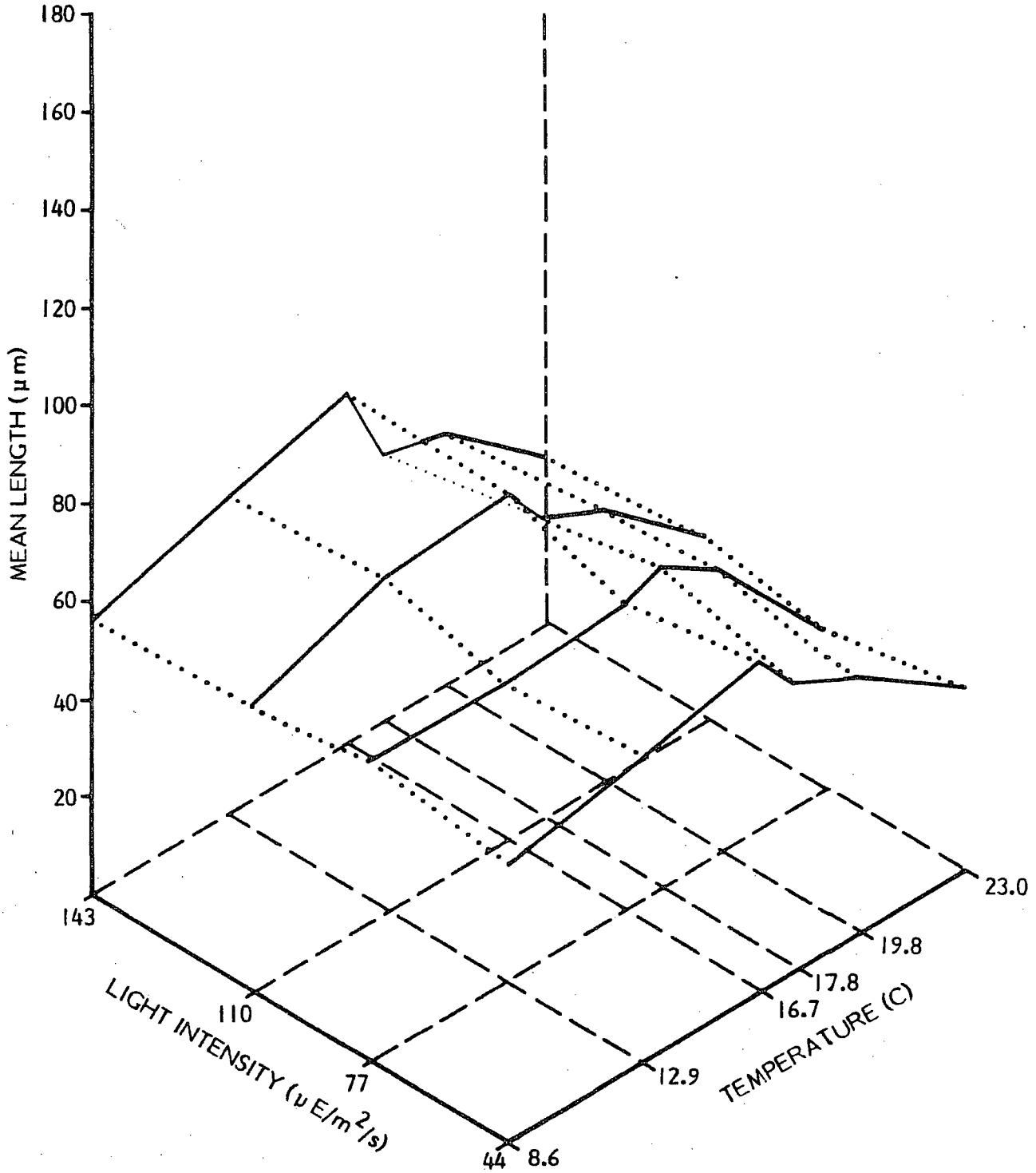


FIGURE 3.7-6

MEAN FILAMENT LENGTH VERSUS TEMPERATURE
AND LIGHT INTENSITY AT DAY 2: BULL KELP
GAMETOPHYTE OPTIMUM TEMPERATURE FOR GROWTH AND FERTILITY

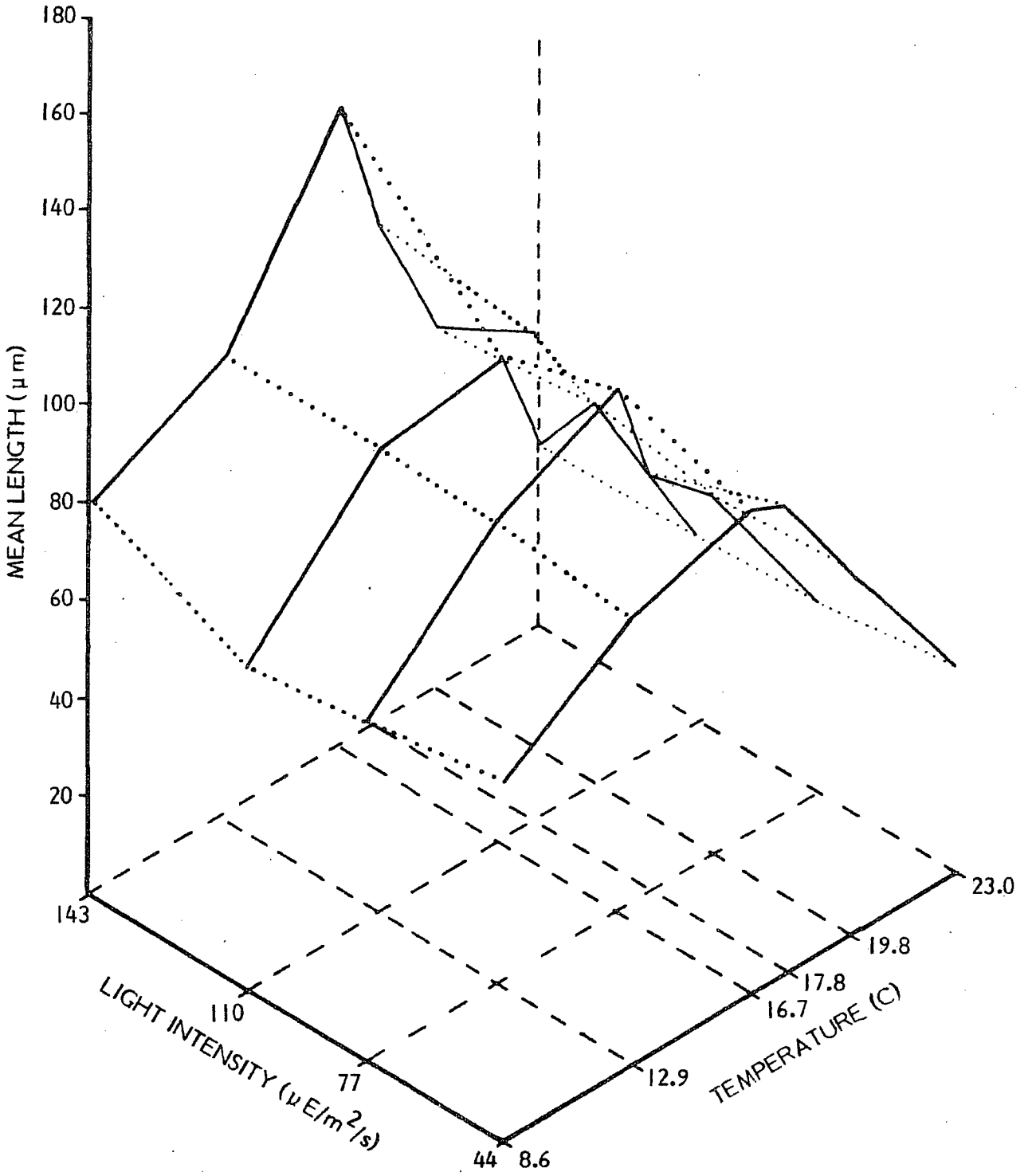


FIGURE 3.7-7

MEAN FILAMENT LENGTH VERSUS TEMPERATURE
AND LIGHT INTENSITY AT DAY 4: BULL KELP GAMETOPHYTE
OPTIMUM TEMPERATURE FOR GROWTH AND FERTILITY

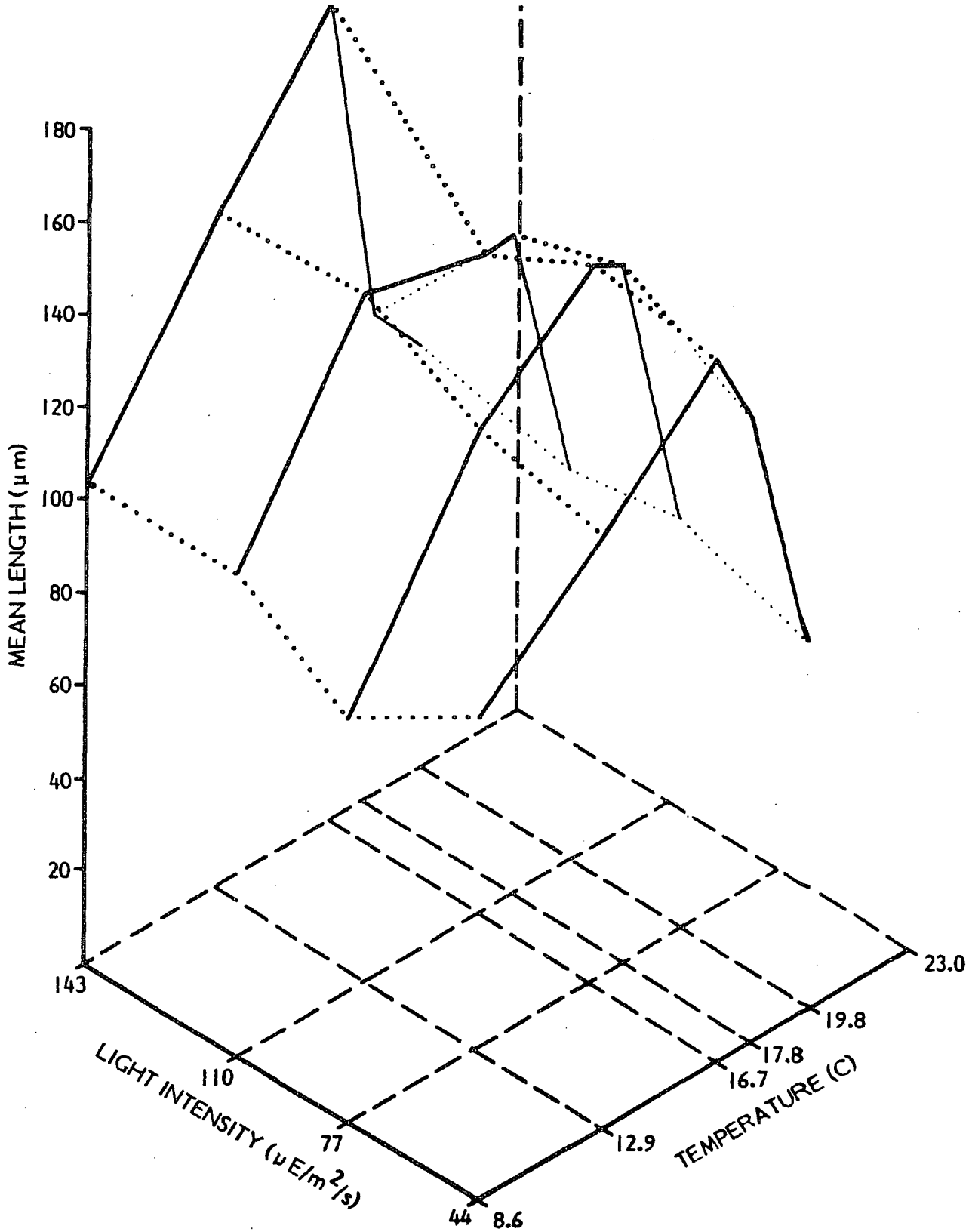


FIGURE 3.7-8

MEAN FILAMENT LENGTH VERSUS TEMPERATURE
AND LIGHT INTENSITY AT DAY 6: BULL KELP GAMETOPHYTE
OPTIMUM TEMPERATURE FOR GROWTH AND FERTILITY

No measurements were made in the 23 C groups.

TABLE 3.7-10

FERTILITY AT DAYS 10 AND 12:
BULL KELP GAMETOPHYTE OPTIMUM TEMPERATURE
FOR GROWTH AND FERTILITY

		DAY 10			DAY 12		
Temperature (C)	Light Intensity ($\mu E/m^2/s$)	Percentage Fertile Females	$\bar{X}$ No. Eggs/Fertile Female	Percentage Fertile Males	Percentage Fertile Males	$\bar{X}$ No. Eggs/Fertile Female	Percentage Fertile Males
	143	0		50	2	1.0 (0.0)	62
8.6	110	0		22	0		34
	77	0		4	0		8
	77	0		10	4	1.0 (0.0)	52
	143	0		58	22	1.2 (0.4)	70
12.9	110	0		48	14	1.3 (0.5)	74
	77	0		10	4	1.0 (0.0)	52
	44	2	1.0 (0.0)	6	0		4
	143	6	1.0 (0.0)	100	98	3.4 (1.3)	100
16.7	110	12	1.0 (0.0)	94	62	1.8 (1.2)	96
	77	2	1.0 (0.0)	76	80	2.3 (1.0)	100
	44	0		4	0		8
	143	76	1.7 (0.7)	100	90	2.1 (1.1)	100
17.8	110	56	1.5 (0.8)	98	84	2.4 (1.1)	100
	77	32	1.2 (0.4)	92	82	2.8 (1.0)	100
	44	48	1.7 (0.9)	92	76	2.2 (1.0)	100

TABLE 3.7-11

CHI-SQUARE CONTINGENCY TABLE COMPARING FEMALE FERTILITY WITH LIGHT INTENSITY AT 17.8 C ON DAY 10: BULL KELP GAMETOPHYTE OPTIMUM TEMPERATURE FOR GROWTH AND FERTILITY

The groups are significantly different at $p = 0.005$.

	Light Intensity ($\mu E/m^2/s$)				
	44	77	110	143	Total
Fertile	24	16	28	38	106
Non-fertile	26	34	22	12	94
Total	50	50	50	50	

$\chi^2 = 15.086$, 3df

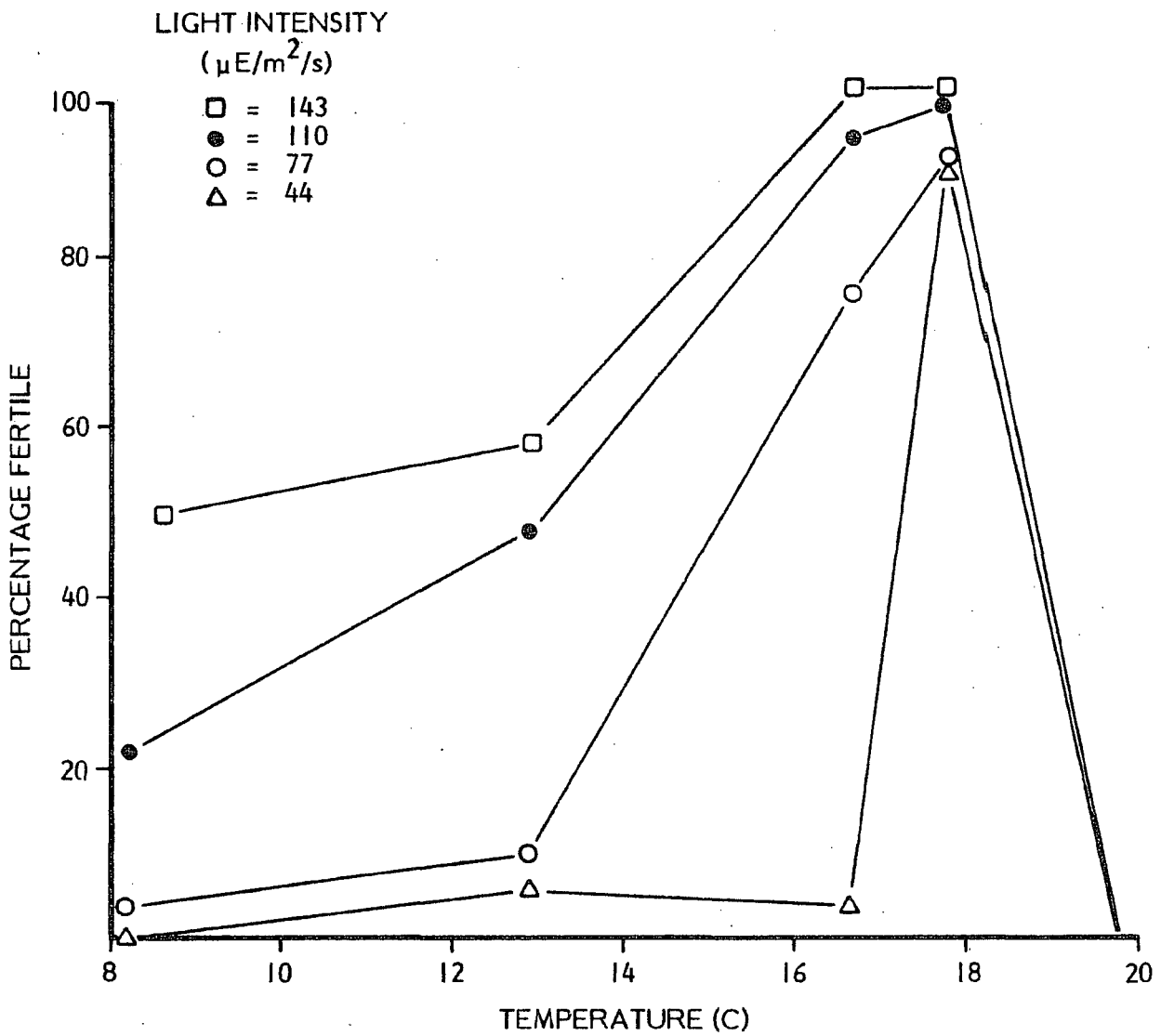


FIGURE 3.7-9

PERCENTAGE FERTILE MALE PLANTS AT DAY 10:
BULL KELP GAMETOPHYTE OPTIMUM TEMPERATURE
FOR GROWTH AND FERTILITY

Percentage fertility was zero in all 19.8 C treatments.

(FIGURE 3.7-10, TABLE 3.7-10). The mean number of eggs per fertile female plant was less than 2 for all treatments. The effect of light on fertility at 17.8 C on day 10 was highly significant (see TABLE 3.7-11). By day 12, there was a general increase in fertility and the trends seen at day 10 were more pronounced. In male plants, fertility was at or near 100 percent in the 16.7 and 17.8 C treatments except for the 44 $\mu E/m^2/s$ dish at 16.7 C which exhibited a very low level of fertility (FIGURE 3.7-11). In general, fertility decreased with decreasing temperature except for the lowest light intensity treatments. These trends were generally paralleled in the female fertility data although the absolute values were lower (FIGURE 3.7-12). An exception is provided by the highest light intensity group in which relatively higher fertility was evident at 16.7 C. However, the possible light effect is not consistent in the intermediate light intensity groups. The mean number of eggs per fertile female plant appeared to increase in proportion to the percentage of fertile females in the sample. The reader should keep in mind the fact that no fertility was observed in the 19.8 C treatment dishes during the course of this experiment. This indicates that there is an extremely abrupt temperature effect on fertility in the 17.8-19.8 C range.

In an experiment on growth and sexuality of bull kelp gametophytes, Vadas (1972) also found growth at 20 C with an absence of fertility. He concluded that fertility of female gametophytes was not significantly affected by temperatures of 5, 10 and 15 C and that light intensity was the most important factor controlling fertility. Results of this experiment demonstrate inhibition of female fertility at low light intensity and at lower temperatures. It is possible that the bull kelp used for this experiment was more sensitive to colder temperatures since the Diablo Canyon area is close to the southern distribution limit for the species. Cultures used by Vadas were from the coast of Washington.

Lünning and Dring (1972) observed a lack of fertility for Laminaria saccharina at 20 C. Lünning and Neushul (1978) found similar inhibition of fertility at 20 C for southern California Laminariales species (Santa Barbara area) but for central California species (from the San Francisco area) fertility was absent at 17 C

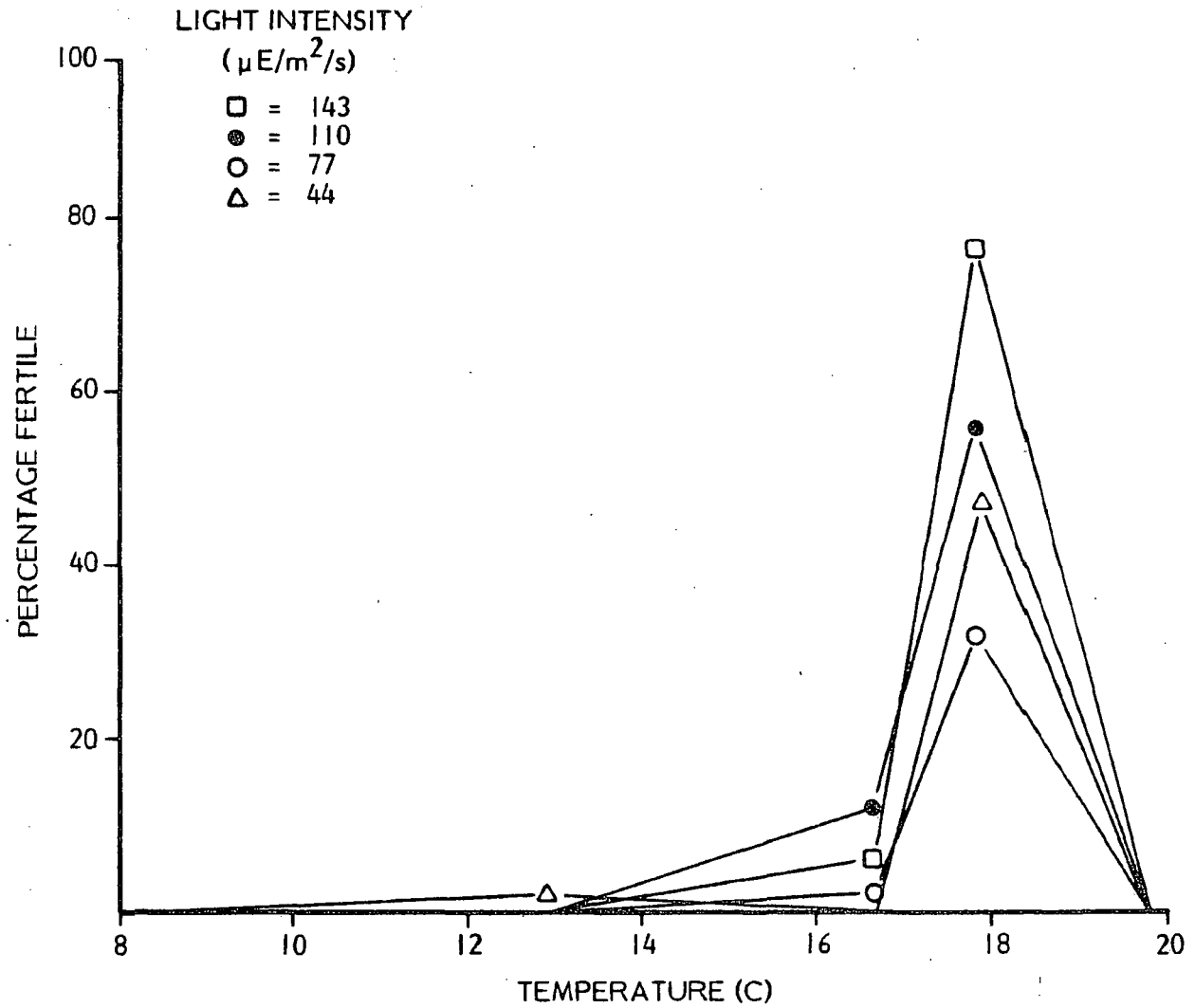


FIGURE 3.7-10

PERCENTAGE FERTILE FEMALE PLANTS AT DAY 10:
BULL KELP GAMETOPHYTE OPTIMUM TEMPERATURE
FOR GROWTH AND FERTILITY

Percentage fertility was zero in all 19.8 C treatments.

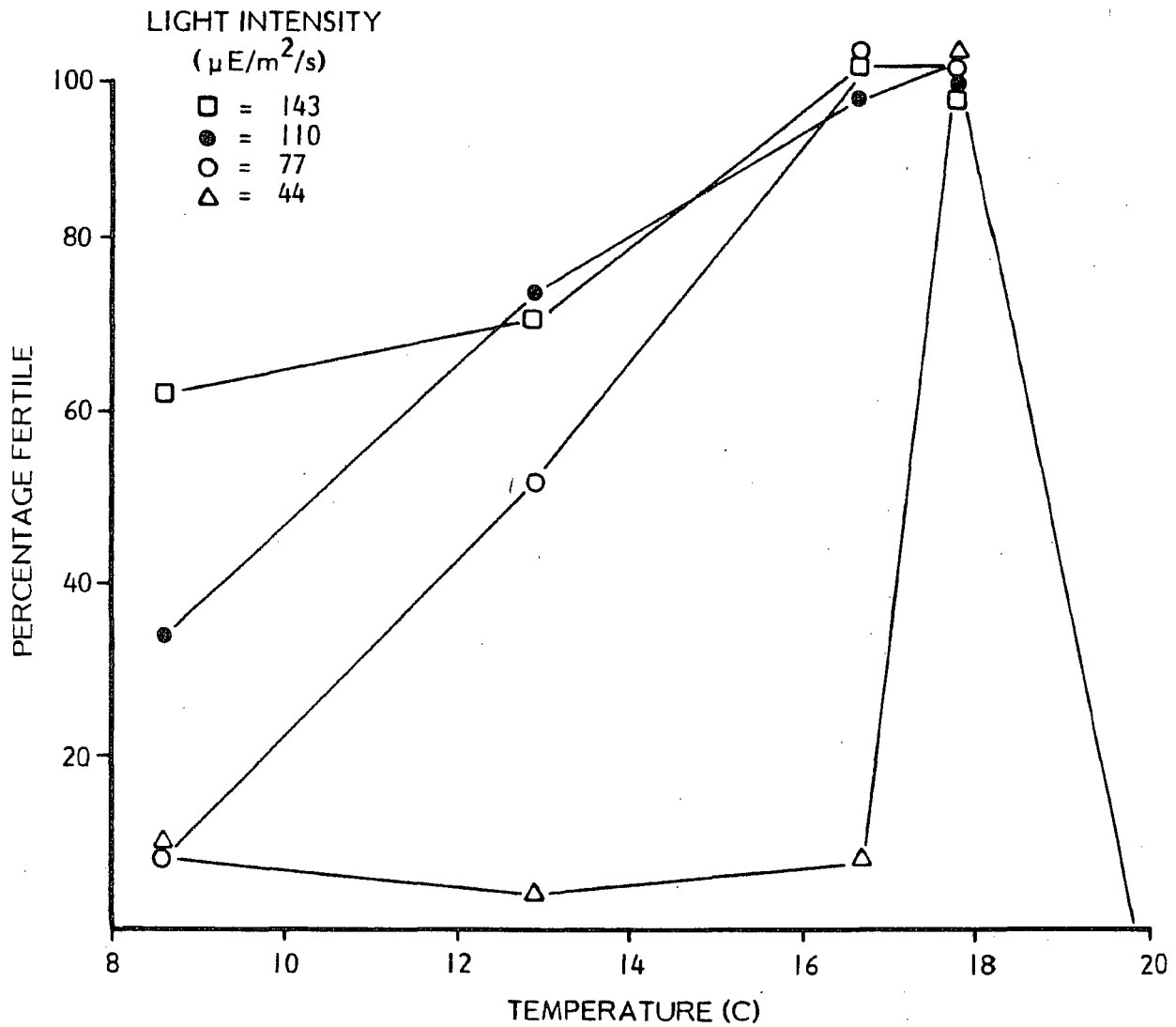


FIGURE 3.7-11

PERCENTAGE FERTILE MALE PLANTS AT DAY 12:
BULL KELP GAMETOPHYTE OPTIMUM TEMPERATURE
FOR GROWTH AND FERTILITY

Percentage fertility was 100 in all 17.8 C treatment
and zero in all 19.8 C treatments.

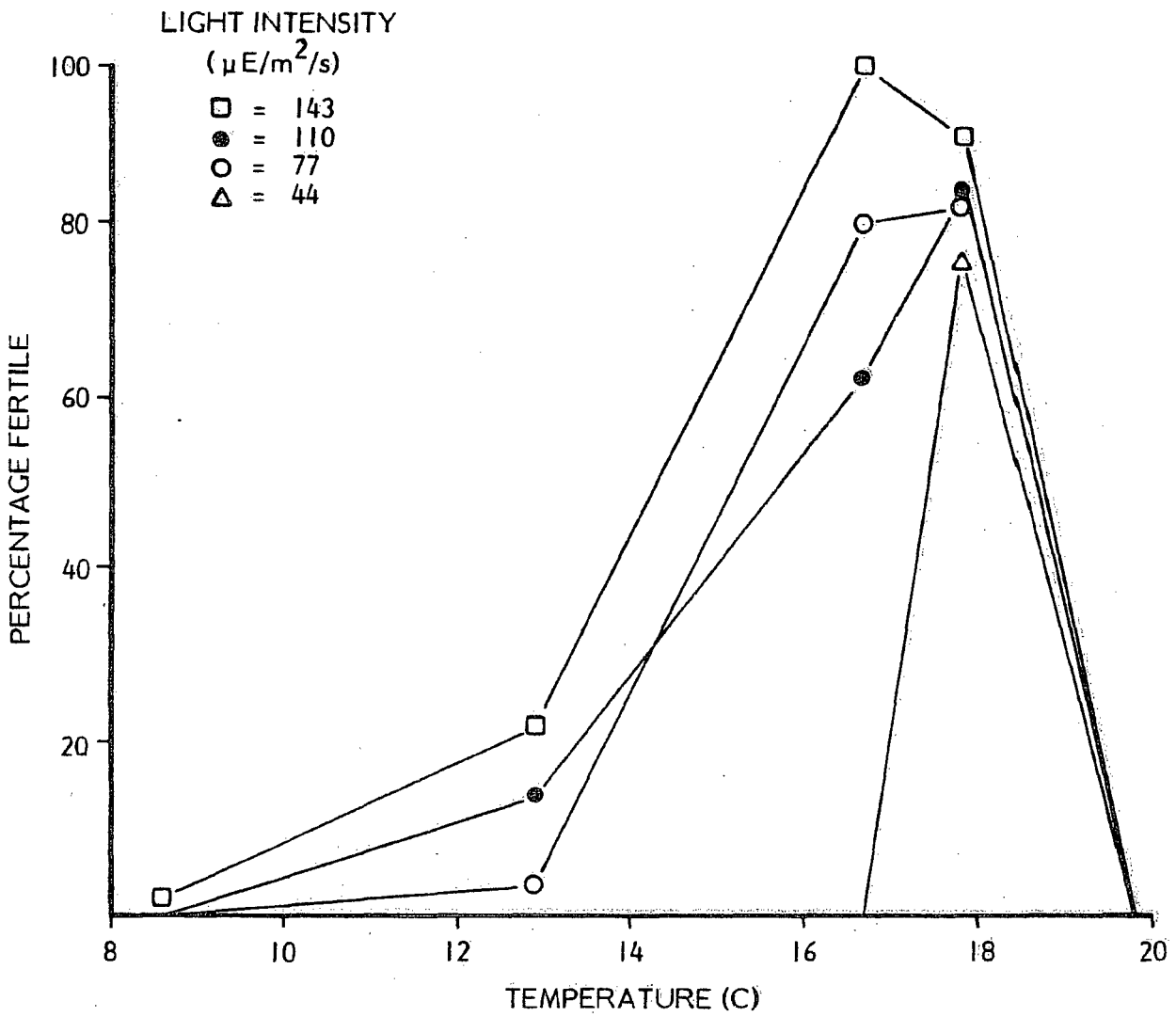


FIGURE 3.7-12

PERCENTAGE FERTILE FEMALE PLANTS AT DAY 12:
BULL KELP GAMETOPHYTE OPTIMUM TEMPERATURE
FOR GROWTH AND FERTILITY

Percentage fertility was zero in all 19.8 C treatments.

TABLE 3.7-12

SPOROPHYTE DEVELOPMENT DATA AT DAYS 10 AND 12:
BULL KELP GAMETOPHYTE OPTIMUM TEMPERATURE
FOR GROWTH AND FERTILITY

		DAY 10			DAY 12		
Temperature (C)	Light Intensity ($\mu E/m^2/s$)	Total No. Females with Sporophytes	Total No. Sporophytes	Mean No. Sporo. /Female	Total No. Females with Sporophytes	Total No. Sporophytes	Mean No. Sporo. /Female
16.7	143	0	0	0.0	4	4	1.0
	110	0	0	0.0	0	0	0.0
	77	0	0	0.0	1	1	1.0
	44	0	0	0.0	0	0	0.0
17.8	143	7	11	1.6	11	12	1.1
	110	0	0	0.0	2	3	1.5
	77	0	0	0.0	1	1	1.0
	44	0	0	0.0	0	0	0.0

after two weeks. Peak fertility for all gametophytes occurred at 12 C as opposed to 17.8 C for this experiment. Although different species were used in these experiments, the inhibition of fertility at or near 20 C is remarkably consistent for all of them. This inhibition probably represents an upper thermal limit to growth because a wide variety of both light quality and intensity was used in these experiments.

Sporophytes

Sporophytes were first observed on day 10 in the 17.8 C 143 $\mu E/m^2/s$ treatment only (TABLE 3.7-12). By day 12 they were also present in cultures at 110 and 77 $\mu E/m^2/s$ at 17.8 C and 143 and 77 $\mu E/m^2/s$ at 16.7 C. Sporophytes were not observed during the experiment at either 8.6 or 12.9 C. Although there appears to be a definite light intensity and temperature effect, with a peak of sporophyte production at 17.8 C, 143 $\mu E/m^2/s$, the duration of the experiment was too short to provide sufficient data for this last developmental stage.

3.7.2.3 DISCUSSION

The optimum temperature for growth of bull kelp gametophytes from the Diablo Canyon area is in the range of 12.9-17.8 C (55.2-64.0 F) and probably has a peak near the upper end of this range at higher light intensities. Above 17.8 C (64.0 F) there is a sharp decline in growth. At 23.0 C (73.4 F), no growth was observed.

Fertility of both male and female plants showed a more marked optimum at 16.7-17.8 C (62.1-64.0 F) and no fertility at 19.8 C (67.6 F) and above. Light was shown to have a significant effect on fertility at most temperatures tested in this experiment in that fertility increased markedly with increasing light intensity.

The initial production of sporophytes was greatest in the treatment with highest fertility, as would be expected. The duration of this experiment was not

sufficient to completely characterize the effects of temperature on this developmental stage, however.

In the experimental conditions where suboptimal growth and fertility were observed, it is possible that over a period of time longer than this experiment was conducted, sufficient growth would eventually occur to permit comparable rates of fertility. This would not be true at 23.0 C (73.4 F) where no growth was observed throughout the experiment.

3.7.3 SPOROPHYTE GROWTH

Bull kelp sporophyte populations form dense subtidal beds in near-shore areas of Diablo Canyon. The populations are of special interest in that they constitute nearly the southern-most habitat occupied by the species in the eastern Pacific. The plants themselves, capable of attaining lengths of 60 ft and more, are subject to the accompanying depth-dependent thermoclines (commonly warm surface water and cooler water below).

The purpose of the experiment was to determine the possible long-term effects of elevated seawater temperatures from the power plant discharge on bull kelp populations in and near Diablo Cove. To this end a long-term (44 day) growth experiment was designed to determine the optimum temperature for growth.

3.7.3.1 METHODS

A summary description of the laboratory methods employed in the investigations into optimum temperature for growth of bull kelp sporophytes is presented below. For a more detailed description of the laboratory methods see APPENDIX B (Procedure 311) and protocol see APPENDIX C (experiment 11b).

Preliminary Studies

Juvenile bull kelp sporophytes are available in the field only in the spring months, and therefore laboratory cultured plants were used in preliminary

temperature tolerance studies. These cultures were initiated from field-collected sori, the spores being released onto 1/4 in. nylon line in the laboratory, with subsequent gametophytic growth, gametogenesis, fertilization and sporophyte induction all occurring on these lines while held in the raceways. Cultures were maintained pre-experimentally at approximately 12 C, under continuous illumination of 130-160 $\mu E/m^2/s$.

Ten small sporophytes were introduced into each of five temperature treatments (12, 15, 17, 19 and 21 C). An additional ten were held at ambient temperatures to serve as a control group. At four day intervals for 12 days the total length of each plant was measured and recorded. Substantial growth (683 percent increase after 12 days at 12 C) occurred at lower temperatures; but negative growth rates occurred at 19 and 21 C, indicating an adverse reaction to those temperatures.

Based on these preliminary results, target temperatures for the present experiment were planned at 2 C increments between 10 C (the minimum temperature attainable in the raceways for prolonged periods) and 20 C (the probable maximum temperature at which juvenile sporophytes might survive for 44 days), with the exclusion of 14 C. Thus, original target temperatures included 10, 12, 16, 18 and 20 C. An ambient treatment was added to serve as a control. Several weeks into the experiment, a 19 C treatment was initiated in the 20 C tank after all of those plants had died.

Procedure

Over 200 juvenile sporophytes were collected by divers on 1 May 1979, from depths of 10 to 25 ft in north Diablo Cove. Holdfasts, which on young plants are small discoid pads, were carefully scraped from the substrate. Plants were held in collecting bags until the divers returned to the boat, where they were transferred to large tubs of seawater for transport to the laboratory. In the laboratory, the plants were held in 15 x 3 x 2 ft flow-through raceways (for a detailed description see PGandE 1977, 1979a), supplied with ambient seawater. Continuous (24 h) illumination was provided by overhead banks of 25 W Duro-Test Ultra-High-Output Vita-Lights.

Plants were held in the raceways for three days prior to the start of the experiment. During this time each plant was inspected for wounds sustained prior to or during collection and damaged plants were discarded.

Individuals were sorted into two size classes (10-20 cm and 20.1-25 cm). Twenty individuals from the smaller size class and ten from the larger size class were haphazardly assigned to each temperature treatment. Each individual was then tagged and numbered haphazardly within each treatment group and size class. In the raceways, plant holdfasts were attached to submerged PVC racks by means of plastic "cafe clips".

A temperature adjustment period was carried out to avoid the adverse effects of thermal shock caused by transfer of plants from ambient to significantly higher experimental temperatures. Thus, in transferring plants to raceways of successively higher temperatures, increments of temperature increase did not exceed 2 C in any 8 h period.

Plants were introduced into their respective experimental raceways on three consecutive days: ambient and 10 C treatments were initiated on May 4, 12 and 16 C treatments on May 5, and 18 and 20 C treatments on May 6. Immediately prior to introduction into the experimental raceway, each plant was photographed, and the following observations and measurements were made and recorded: total length, stipe length, presence or absence of pneumatocysts and number of splits in the blade. Additionally, each plant was punctured, with a sewing needle, 23 mm above the base of the blade (33 mm above the base for group A plant's after 32 days in the ambient, 10, 12 and 16 C treatments). Migration of these minute puncture wounds along the blade served as an indication of blade elongation, and the measurement of such, for the purposes of this experiment, was termed the "growth increment measurement." Careful observation showed that these punctures did not have an adverse effect on surrounding tissue. The above measurements were repeated for each plant at 4 d intervals throughout the experiment.

The plants within each treatment were divided into three sampling groups of 10 plants each (see FIGURE 3.7-13): group A plants were to be carried through the experiment for 44 days; group B were removed after 28 days and group C were removed after 12 days (group C plants were from the larger size class of field-collected material whereas groups A and B plants were from the smaller size class). Upon removal from the tank, plants within each sample group were photographed, weighed (wet), dried at approximately 100 C and weighed again.

Light intensities above each raceway were measured at approximately eight d intervals throughout the experiment. Measurements were made using a Li-Cor quantum meter (model LI-185) equipped with a surface sensor. For each reading the sensor was placed approximately 12 cm above the water surface (61 cm above the raceway bottom); readings were made at nine pre-designated positions above each raceway.

Water samples were taken from each raceway every eight days; these were analyzed for pH, salinity, nitrate, phosphate, and ammonia content.

Raceways were cleaned periodically as described in the experiment procedure (see APPENDIX B).

Water temperatures were monitored and recorded hourly on paper tape by the Autodata Nine computer. Temperature probes were positioned at opposite ends of each racway, providing two simultaneous temperature readings for each treatment. All temperature data were transcribed manually from the paper tapes.

3.7.3.2 RESULTS

The temperature data for this experiment are summarized in TABLE 3.7-13. The mean and standard deviation values are given for each probe, calculated over the 44-day period (except for the 20.0 C treatment which was terminated on 18 May and the 18.8 C treatment which was started on 26 May), the mean value of the two probes in each raceway, and the minimum and maximum temperatures occurring in each raceway. The data reveal that there exists a

TREATMENT	GROUP	NUMBER OF DAYS GROWTH											
		00	04	08	12	16	20	24	28	32	36	40	44
10C	A	●	●	●	●	●	●	●	●	●	●	●	●
	B	●	●	●	●	●	●	●	●				
	C	●	●	●	●								
12C	A	●	●	●	●	●	●	●	●	●	●	●	●
	B	●	●	●	●	●	●	●	●				
	C	●	●	●	●								
16C	A	●	●	●	●	●	●	●	●	●	●	●	●
	B	●	●	●	●	●	●	●	●				
	C	●	●	●	●								
18C	A	●	●	●	●	●	●	●	●	●	●	●	
	B	●	●	●	●	●	●	●	●				
	C	●	●	●	●								
19C	A	●	●	●	●	●	●	●					
	B	●	●	●	●	●	●	●					
	C	●	●	●	●								
20C	A	●	●	●	●								
	B	●	●	●	●								
	C	●	●	●	●								
AMBIENT	A	●	●	●	●	●	●	●	●	●	●	●	●
	B	●	●	●	●	●	●	●	●				
	C	●	●	●	●								

FIGURE 3.7-13

SAMPLING DIAGRAM FOR EXPERIMENTAL OBSERVATIONS:
BULL KELP SPOROPHYTE GROWTH

Dots indicate observation and measurement completion.

TABLE 3.7-13

STATISTICAL SUMMARY OF TEMPERATURE DATA:
BULL KELP SPOROPHYTE TEMPERATURE
OPTIMUM FOR GROWTH

Target Temperature	Probe	Mean	Standard Deviation	Mean of Probes A and B	Maximum	Minimim
Ambient	A	12.6	1.48	12.8	16.9	9.9
	B	12.9	1.49			
10	A	10.0	0.50	10.2	13.0	9.5
	B	10.3	0.58			
12	A	11.7	0.17	11.8	14.5	11.1
	B	12.0	0.21			
16	A	16.0	0.32	15.9	18.2	10.9
	B	15.8	0.32			
18	A	17.9	0.24	18.0	19.4	13.9
	B	18.1	0.18			
19	A	18.7	0.08	18.8	19.1	18.3
	B	18.9	0.10			
20	A	19.9	0.32	20.0	21.8	19.3
	B	20.1	0.28			

temperature gradient of 0.2-0.3 C within each raceway, the lower temperatures occurring near the influent port (probe A) and the higher near the effluent port (probe B). This gradient is largely caused by heating of the water by the overhead lights as it flows down the raceway. As early as day 2, it was evident that the 20.0 C treatment plants would not survive long, so preparations were made to replace it with the 18.8 C treatment group which was started on 26 May so that day 0 of the 18.8 C treatment is equivalent to day 20 of the 18.0 C treatment.

Minimum and maximum temperature values for each treatment show a considerable range of temperatures experienced within each raceway. Excluding the ambient temperature treatment (for which there was no temperature control), the two treatments that experienced the greatest range of temperatures were 15.9 and 18.0 C, showing a range of 7.3 and 5.5 C, respectively. Closer inspection reveals that, for the 15.9 C treatment, temperature deviated 5.0 C below the mean, and only 2.3 C above. Similarly, in the 18.0 C treatment, the temperature deviation of greater magnitude occurred in the negative (colder) direction. These minima were recorded for one hourly reading only, during a boiler malfunction.

Deviations in the positive (warmer) direction were greatest for the low-temperature treatments (10.2, 11.8 and 15.9 C). Of the high temperature treatments, the 18.0 and 20.0 C tanks experienced the greatest positive deviations. The 18.8 C treatment exceeded the target temperature by only 0.1 C.

Light intensity measurements are summarized in TABLE 3.7-14. Values represent maximum and minimum intensities observed above each raceway. Over the 44-day period, considerable variability in light intensity was measured both between raceways at a single time, and above a single raceway at different times. The overall minimum and maximum intensities measured (85 and 165 $\mu E/m^2/s$) differ approximately by a factor of two.

The 10.2 and 11.8 C treatments appear from inspection of TABLE 3.7-14 to have been exposed to higher intensities than the other treatments. These differences

TABLE 3.7-14

MINIMUM AND MAXIMUM LIGHT INTENSITY VALUES:
BULL KELP SPOROPHYTE OPTIMUM TEMPERATURE FOR GROWTH

The 18.8 C treatment was started on 26 May and the 20.0 C treatment was terminated on 18 May.

	TREATMENT													
	Ambient		10.2 C		11.8 C		15.9 C		18.0 C		18.8 C		20.0 C	
Date	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
14 May	126	142	148	165	152	165	130	142	130	148	–	–	139	152
22 May	129	143	148	168	146	165	128	143	132	148	–	–	–	–
1 June	86	95	101	112	98	111	85	95	88	98	96	103	–	–
11 June	143	147	148	152	144	149	143	147	144	148	146	150	–	–
19 June	93	98	103	113	98	108	90	100	93	96	98	108	–	–

may have had an effect on growth rates. Nitrate-nitrogen and ortho phosphate concentrations in seawater samples taken six times during the experiment from each tank were measured and the data are presented in TABLE 3.7-15. A great deal of variation is evident in these data which may be in part reflective of natural variation in nutrients in the seawater source; however, marked variations were measured between tanks at one sampling period. Despite the wide fluctuations, there does not appear to be any pattern to the variation and it is assumed that the effects on growth, if any, were random through time and tended to cancel out over the length of the experiment. Ammonia-nitrogen and nitrite-nitrogen levels were also analyzed and in all samples were below 0.3 μg/l and 3.0 μg/l, respectively. Seawater pH ranged from 7.52 to 7.97 and salinity from 33 to 34 parts per thousand.

Mean total length values through time for all treatments are shown in FIGURE 3.7-14. At 44 days, plants held at 11.8 C had achieved the greatest lengths, followed by plants from ambient, 10.2 and 15.9 C treatments. Decrease in total length resulting from tissue death and deterioration was observed in plants from the 18.0, 18.8 and 20.0 C treatments.

Regression lines of log total length of total sample populations within each temperature treatment versus time are given in FIGURE 3.7-15. Corresponding regression coefficients, y intercepts, and standard error values are presented in TABLE 3.7-16. From this analysis, growth rates of plants held at ambient, 10.2, 11.8 and 15.9 C were all positive and similar in value. Plants from the 18.0, 18.8 and 20.0 C treatments all exhibited negative rates of increase and these rates differ by a wide margin from each other.

Stipe length data (FIGURE 3.7-16), plotted as log mean stipe length versus time for all treatments, reveal trends identical in character to those observed for total length data as treated in FIGURE 3.7-14. Maximum length was attained by plants at 11.8 C followed by plants from ambient, 10.2 and 15.9 C treatments. Negative growth rates were observed at 18.0, 18.8 and 20.0 C.

TABLE 3.7-15

NITRATE AND PHOSPHATE CONCENTRATIONS IN SEAWATER SAMPLES FROM THE EXPERIMENTAL TANKS: BULL KELP SPOROPHYTE OPTIMUM TEMPERATURE FOR GROWTH

Treatment (C)	Date 5-10 N	5-10 P	5-18 N	5-18 P	5-25 N	5-25 P	6-03 N	6-03 P	6-11 N	6-11 P	6-17 N	6-17 P
10.2	274	50	165	40	919	100	137	30	100	40	300	90
11.8	315	50	494	40	644	50	137	10	200	40	400	70
15.9	384	50	548	30	261	30	178	30	200	10	500	40
18.0	343	30	247	30	178	40	137	30	100	10	300	70
18.8							206	30	100	20	300	10
20.0	329	30	165	30								
Ambient	713	50	384	30	329	30	123	40	100	10	400	40

Concentration in μg/liter

(N = NO_3 nitrogen, P = PO_4 phosphorus).

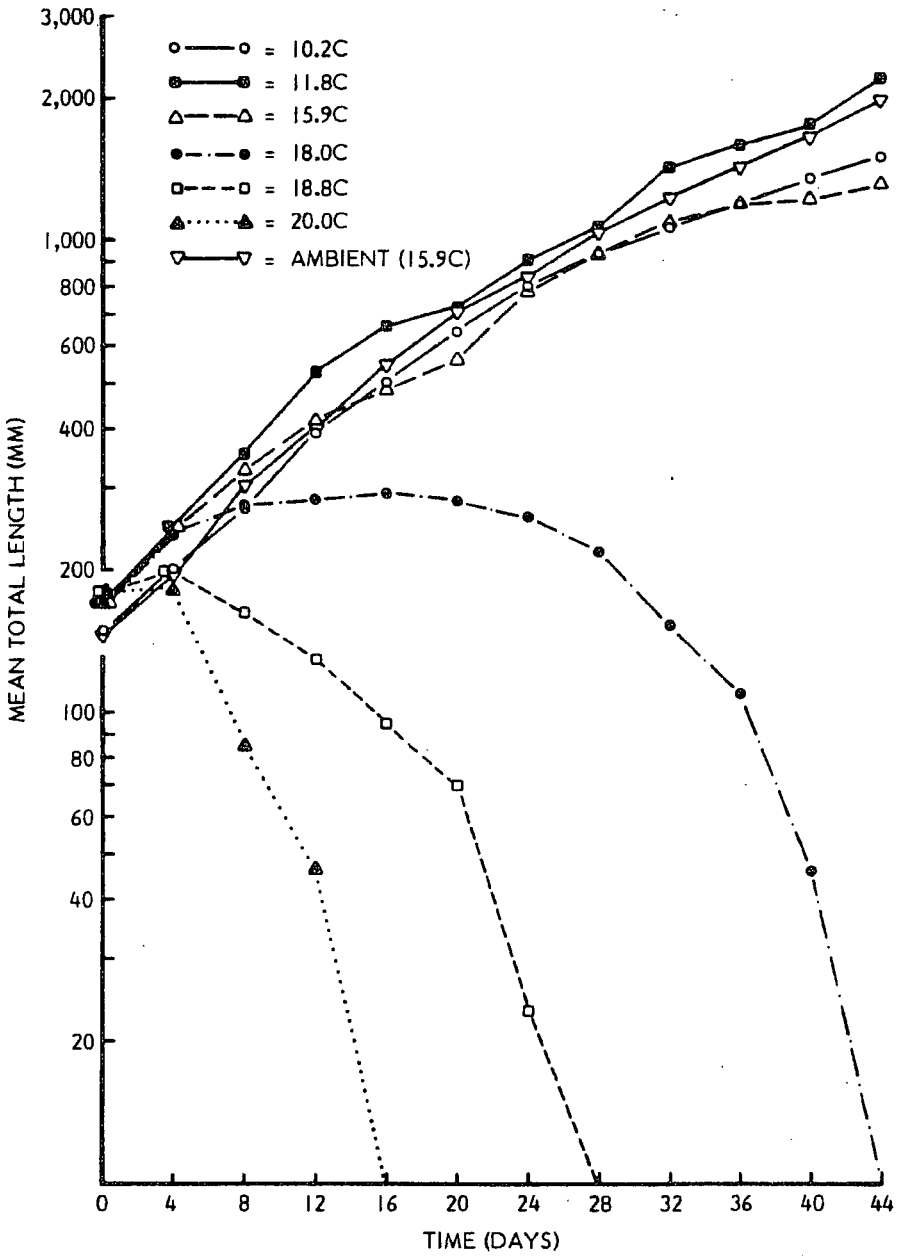


FIGURE 3.7-14

MEAN TOTAL LENGTH VERSUS TIME:
BULL KELP SPOROPHYTE OPTIMUM
TEMPERATURE FOR GROWTH

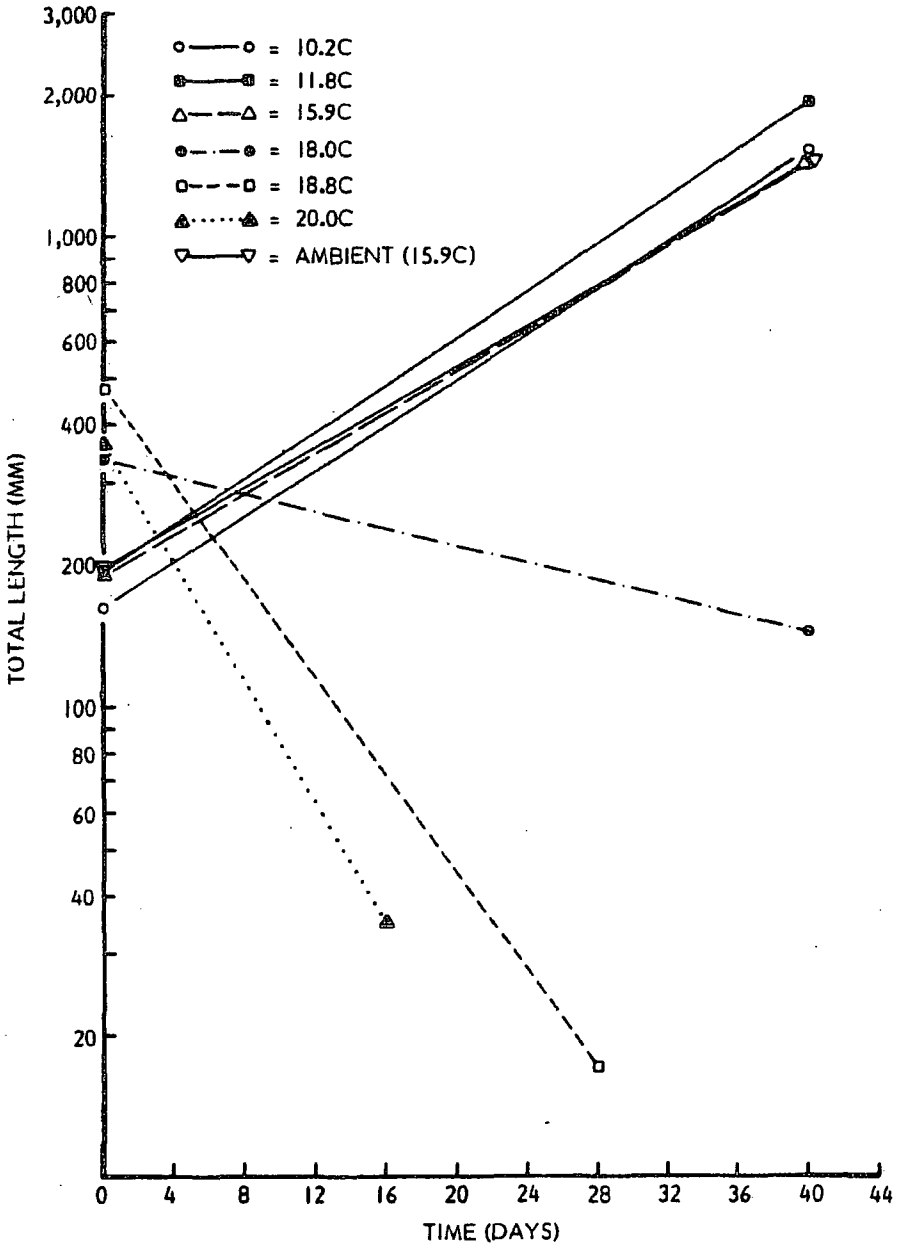


FIGURE 3.7-15

REGRESSION LINES FOR TOTAL LENGTH VERSUS TIME:
BULL KELP SPOROPHYTE OPTIMUM TEMPERATURE FOR GROWTH

TABLE 3.7-16

RESULTS OF REGRESSION ANALYSIS OF TOTAL LENGTH VERSUS TIME FOR EACH TREATMENT: BULL KELP SPOROPHYTE OPTIMUM TEMPERATURE FOR GROWTH

Target Temperature (C)	Regression Coefficient (B) Log_{10}	Intercept Log_{10}	Standard Error of B
Ambient	0.0213	2.30	0.001
10.2	0.0242	2.21	0.001
11.8	0.0249	2.29	0.001
15.9	0.0218	2.28	0.001
18.0	-0.00425	2.53	0.001
18.8	-0.0516	2.68	0.004
20.0	-0.0632	2.56	0.007

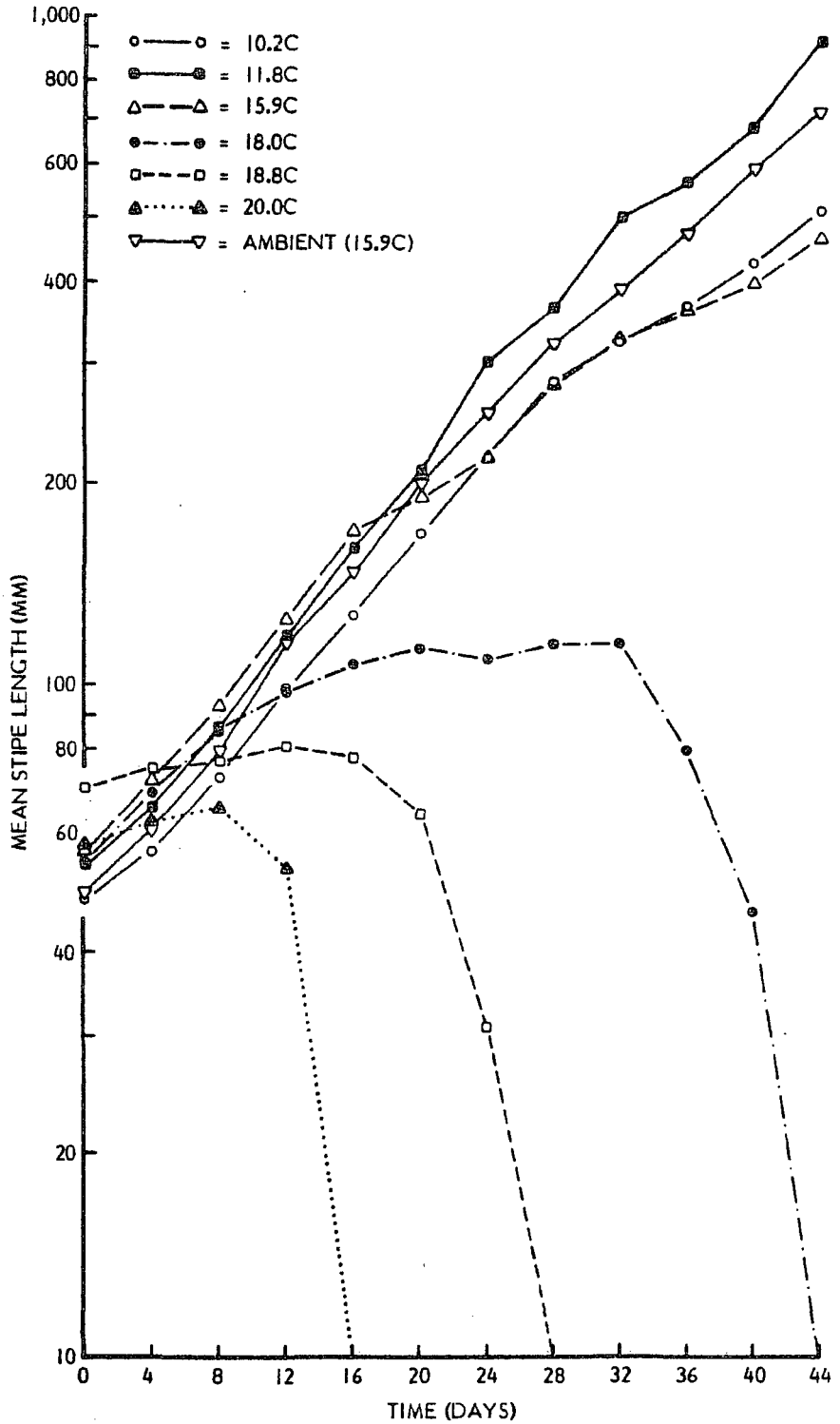


FIGURE 3.7-16

MEAN STIPE LENGTH VERSUS TIME:
BULL KELP SPOROPHYTE OPTIMUM
TEMPERATURE FOR GROWTH

Growth increment data, plotted as log mean increment/4 day versus time for all treatments, are shown in FIGURE 3.7-17. At higher temperatures (18.0, 18.8 and 20.0 C) sharp decreases in growth increment were observed and became zero by days 8, 12 and 24, respectively. It should be noted that because the meristematic region gradually moved into the area marked for these increment measurements, the values decrease with time. Locating the marks further along the blade would have obviated this problem. Because of the high variability in the data, it is not possible to detect any differences in trends between the 10.2, 11.8, 15.9 C and ambient treatments.

Histograms representing wet weight and dry weight measurements are shown in FIGURES 3.7-18 to 3.7-21. The measurements were made after 0, 12, 28 and 44 days growth, and represent non-repetitive, destructive sampling.

Results for both wet weight and dry weight values at 12 days (FIGURE 3.7-19) approximate a normal curve, with the greatest values occurring in the 12 C treatment. At 28 days (FIGURE 3.7-20) we see a departure from the normal curve; the prevailing growth trend (i.e., substantial growth at ambient, 10.2, 11.8 and 15.9 C and lack of such at 18.0, and 18.8 and 20.0 C) is nevertheless apparent. At 44 days, (FIGURE 3.7-21) values approaching a normal distribution are again evident. Most importantly, however, the 11.8 C treatment is seen to have produced the greatest biomass, followed closely by the ambient and 10.2 C treatments. Values for the 15.9 C treatment are declining relative to the former three temperature treatments; and 18.0, 18.8 and 20.0 C all show values of zero (indicating that no plants remained in those treatments at that time).

Information regarding plant mortality in the various treatments is discussed in SECTION 2.7 of this report. It should be noted that 100 percent mortality had occurred at 18.0 C by 40 days, at 18.8 C by 28 days and at 20.0 C by 16 days.

3.7.3.3 DISCUSSION

The optimum temperature for growth of young bull kelp sporophytes was broadly defined in this experiment to be in the range 10.2-15.9 C (50.4-60.6 F). Because

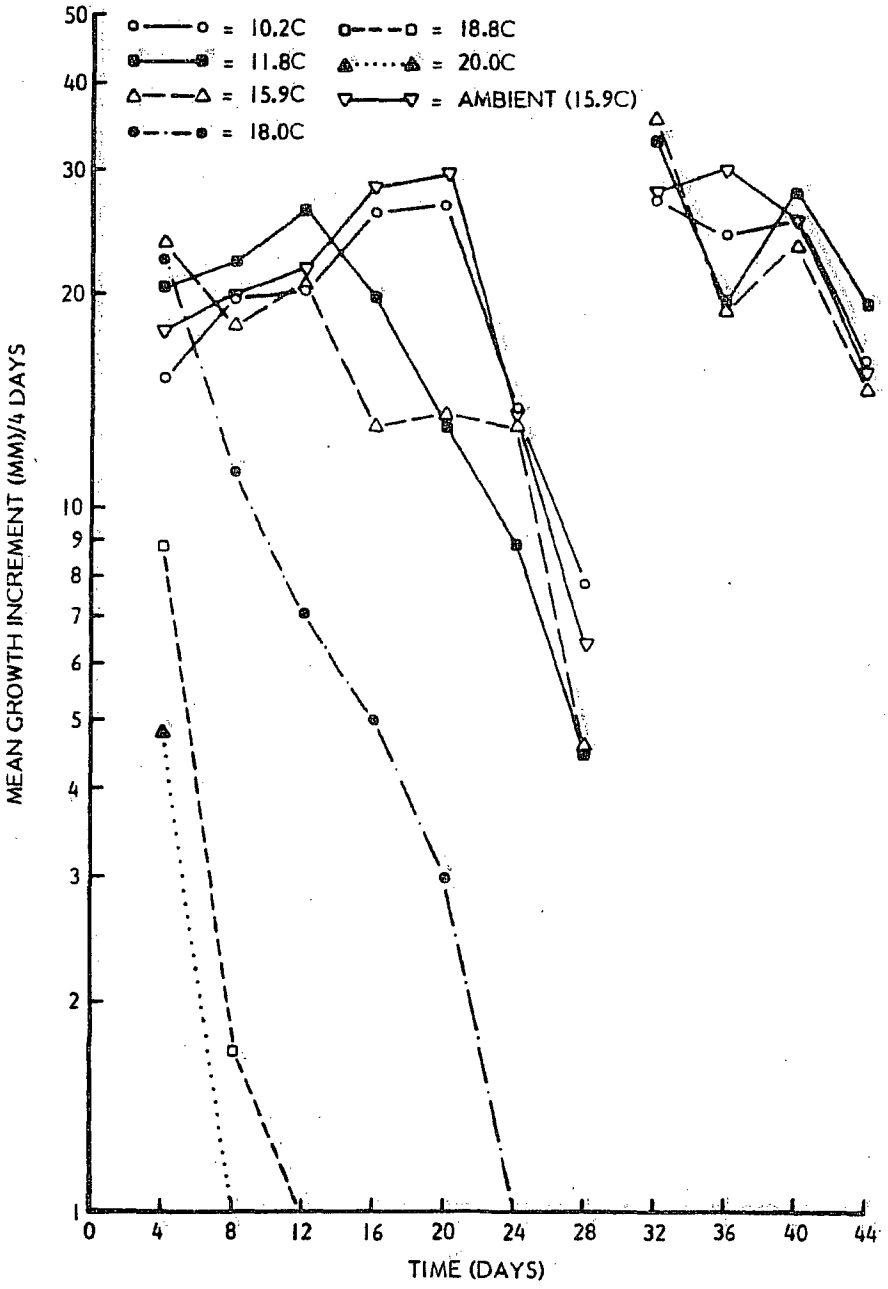


FIGURE 3.7-17

MEAN INCREMENTAL GROWTH VERSUS TIME:
BULL KELP SPOROPHYTE OPTIMUM
TEMPERATURE FOR GROWTH

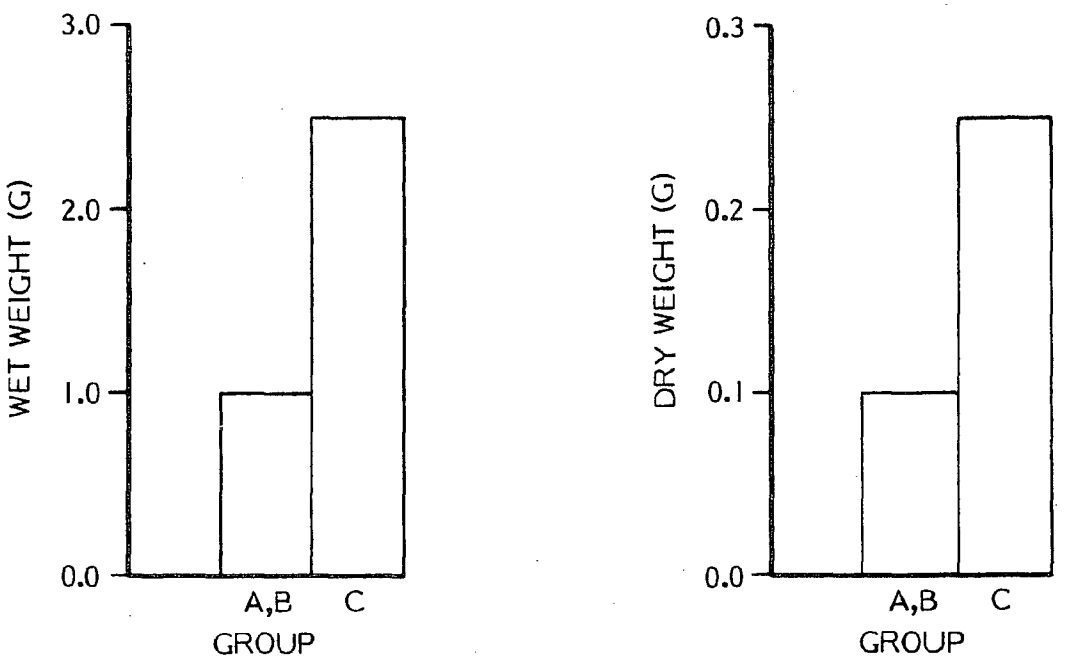


FIGURE 3.7-18

WET AND DRY WEIGHT MEASUREMENTS ON DAY 0:
BULL KELP SPOROPHYTE OPTIMUM
TEMPERATURE FOR GROWTH

A, B refer to plants of smaller size class
C refers to plants of larger size class (see text)

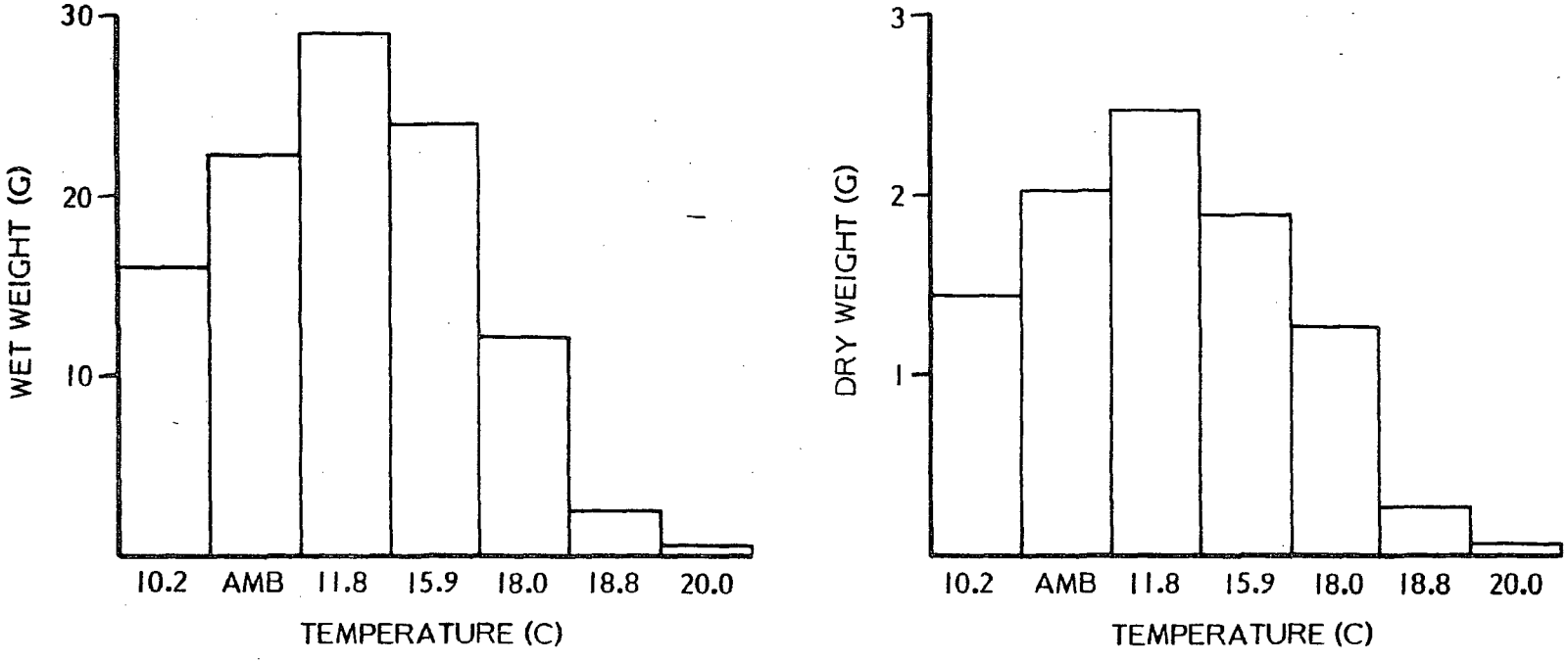


FIGURE 3.7-19

WET AND DRY WEIGHT MEASUREMENTS OF GROUP C PLANTS ON DAY 12: BULL KELP OPTIMUM TEMPERATURE FOR GROWTH

AMB = ambient treatment

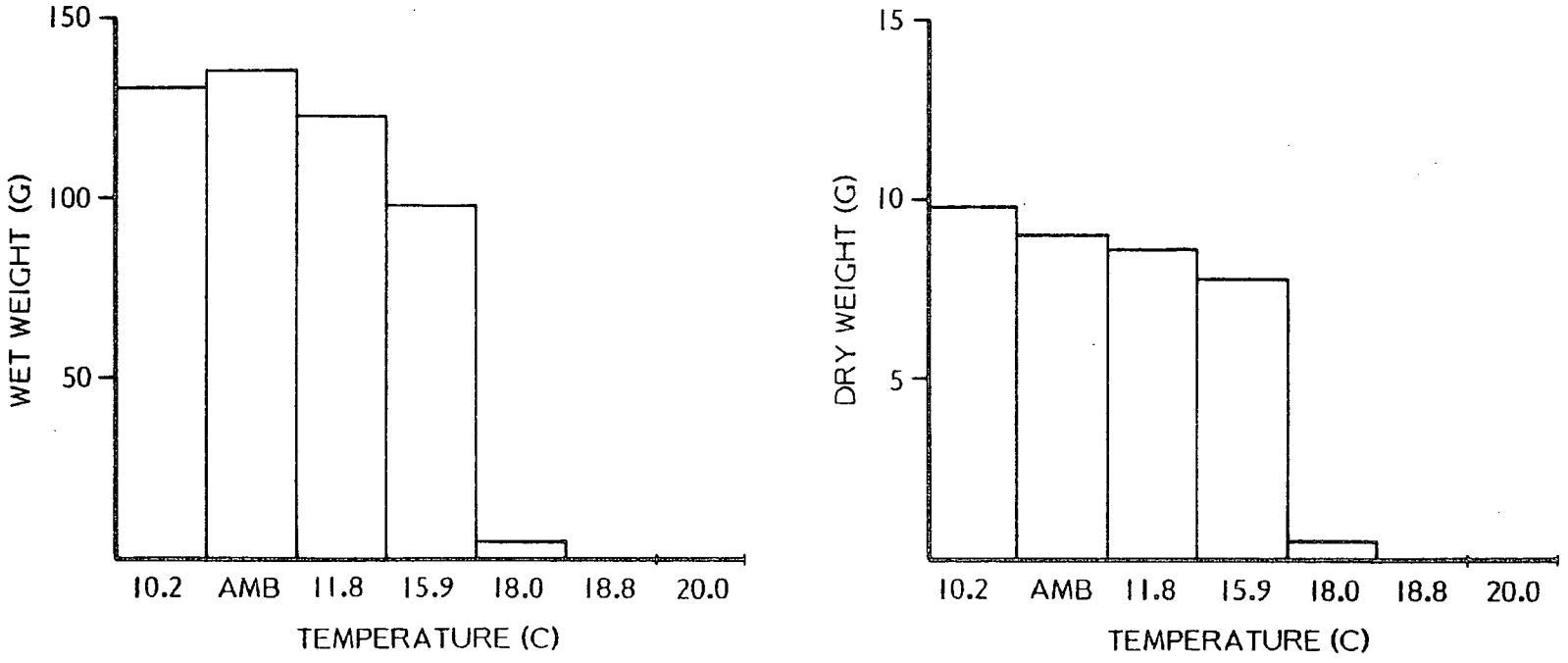
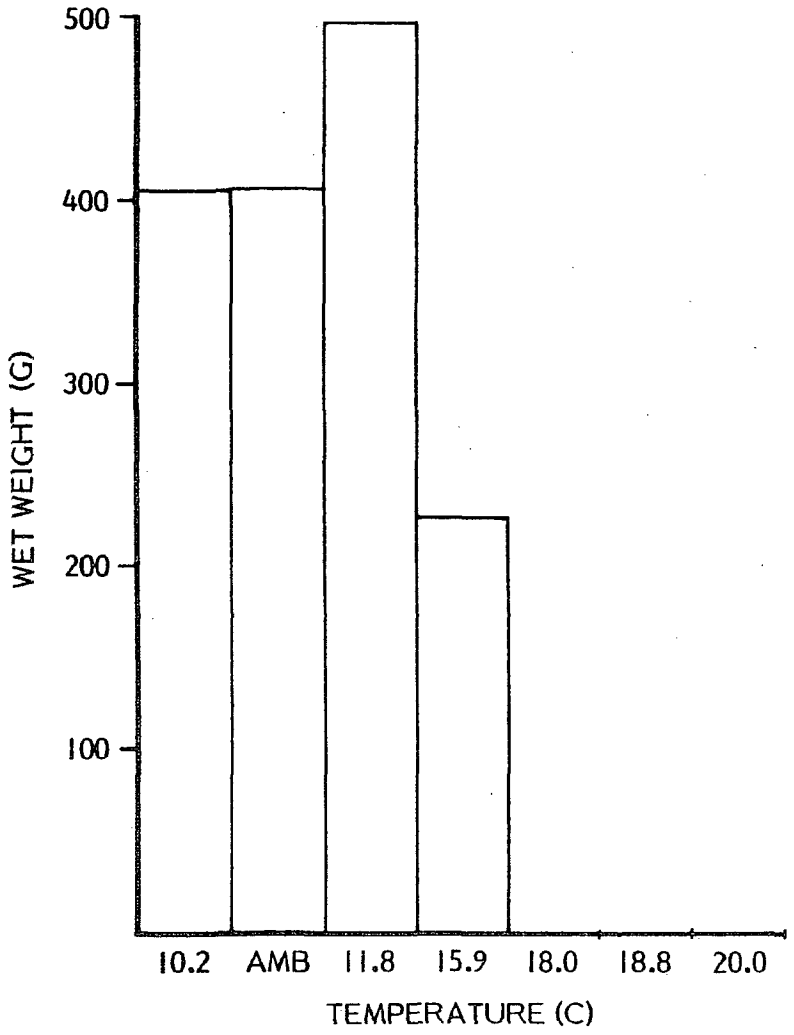


FIGURE 3.7-20

WET AND DRY WEIGHT MEASUREMENTS OF GROUP B PLANTS ON DAY 28: BULL KELP OPTIMUM TEMPERATURE FOR GROWTH

AMB = ambient treatment



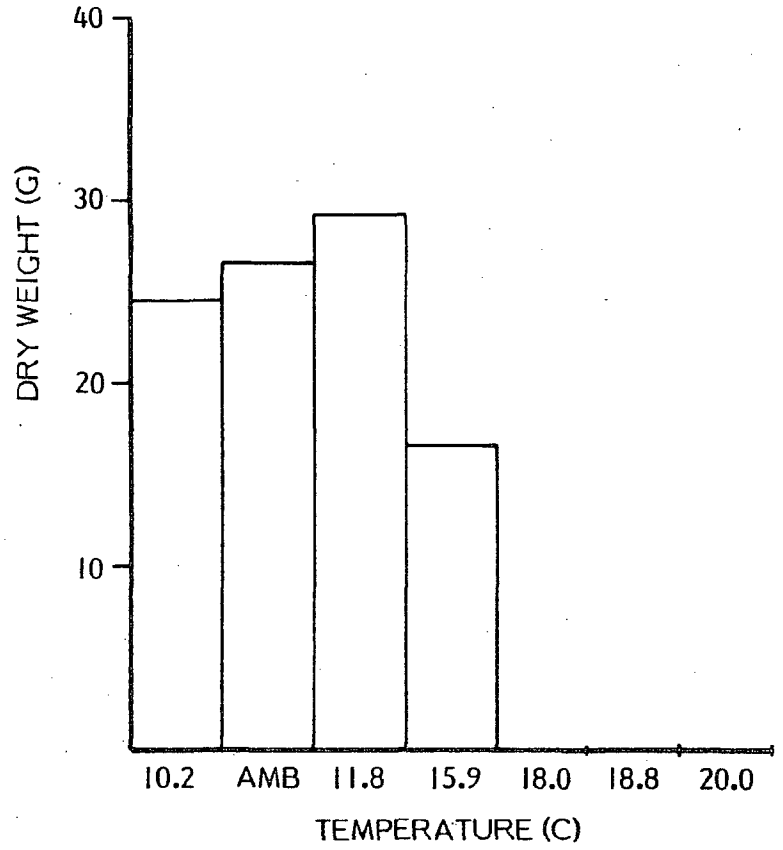


FIGURE 3.7-21

WET AND DRY WEIGHT MEASUREMENTS OF
GROUP A PLANTS ON DAY 44: BULL KELP
SPOROPHYTE OPTIMUM TEMPERATURE FOR GROWTH

AMB = ambi[illegible] treatment

of the inherent variability between individuals within each treatment, it was not possible to statistically demonstrate any growth differences within this range. Visual inspection of the regression analysis on length data and means of biomass data indicate that the optimum may be near 11.8 C (53.2 F). Above 15.9 C (60.6 F), a sharp drop in growth is apparent in that all plants had died by 40 days at 18.0 C (64.4 F), by 20 days at 18.8 C (65.8 F) and by 16 days at 20.0 C (68 F). Caution must be exercised in translating these results to predictions of effects of the power plant. It is possible that the relatively low light levels in the laboratory as compared with those in nature near the ocean surface, may have contributed to mortality at increasing temperatures because the photosynthetic rate was limited by available light so that an excess of respiration resulted, leading to eventual death of the plants. There appears to be little doubt, however, that temperatures of 20 C (68 F) are lethal even over short periods of time.

3.8 TREE KELP (PTERYGOPHORA CALIFORNICA)

The life history of the important species tree kelp (Pterygophora californica) consists of an alternation of a large sporophytic with a microscopic gametophytic generation. The sporophyte is perennial and reproduces annually by means of zoospores that are released into the plankton. The zoospores settle and germinate to produce the gametophytic generation. For a detailed review of the literature on this species, see PGandE (1979b). Sexual reproduction by the gametophytes gives rise to the sporophytes.

In this section, the results of three experiments are presented. The effects of temperature on zoospore germination and early development were studied in the first. In the second, temperature effects on growth and fertility of gametophytes were studied. In the third experiment, temperature effects on growth of sporophytes were investigated.

3.8.1 GAMETOPHYTE GERMINATION AND EARLY DEVELOPMENT

The study objective of this experiment was to determine the minimum, optimum and maximum temperatures for germination and early development of tree kelp zoospores in several light conditions. The purpose was to ascertain the effects of the power plant thermal plume on recruitment of tree kelp gametophytes in Diablo Cove.

3.8.1.1 METHODS

A brief description of the methods employed in the experiment on early development in tree kelp is presented below. For a more detailed description see APPENDIX B, (Procedure 309) and protocol see APPENDIX C (experiment 9b).

Fertile tree kelp sporophytes were collected from an area south of the power plant intake cove using SCUBA on 12 February 1979. They were transported to the laboratory in a cloth dampened with seawater and held in an ambient

raceway until needed for the experiment. Fertile blades from one plant were rinsed briefly in fresh water and then wiped several times with filtered seawater. After drying on a damp towel at 10 C for 15 min and at room temperature for 5 min, they were placed in 10 C filtered seawater and returned to a dark 10 C incubator. Spore release occurred within 10 minutes. After a clean spore suspension was obtained, the proper spore dilution was made. The spores were seeded onto coverslips and allowed to settle for 6 hours in a dark 10 C incubator. Culture dishes were set up as for experiment 9a (see SECTION 3.7.1.1) at target temperatures of 9, 13, 17, 21 and 25 C. Of the four dishes at each temperature, three were exposed to light intensities of 44, 77 and 110 $\mu E/m^2/s$ and one was maintained as a dark control.

Two dishes were placed in an ambient raceway, one at approximately 77 $\mu E/m^2/s$ and the other in the dark (see TABLE 3.8-1). After 24 hours one coverslip was removed from each dish and fixed in 4 percent formalin in seawater. The percentage germination of spores was assessed by observing 25 randomly selected fields of spores. This was repeated after 48 and 72 hours. In a few cases coverslip loss prevented sampling at 48 hours. In general, if germination was greater than 95 percent at 24 or 48 hours, counts were not made at the next sampling. To assess growth, 25 gametophytes were measured after 72 hours and again after one week.

3.8.1.2 RESULTS

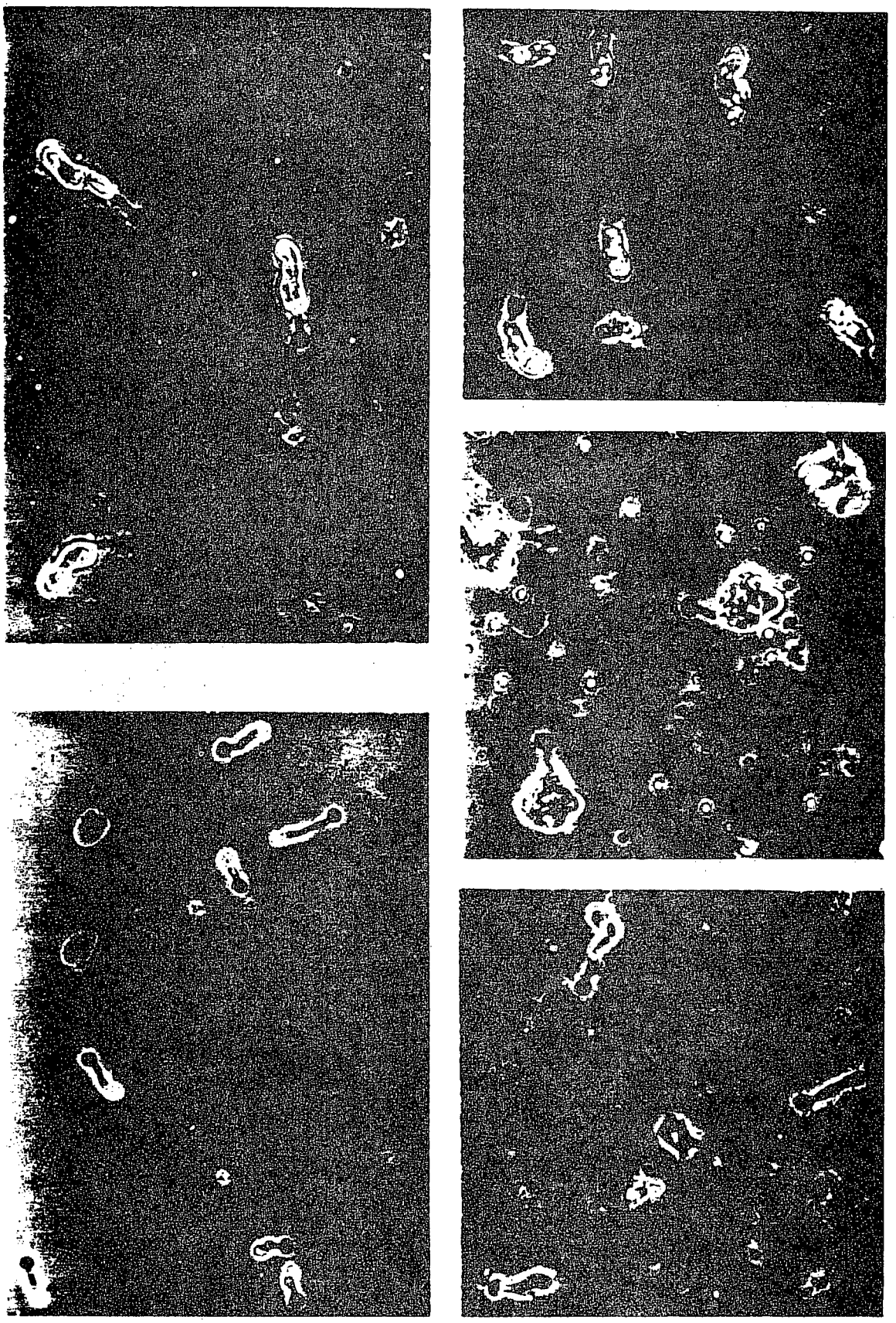
The results presented in this section summarize all the temperature data collected for the experiment and the mean values for germination and growth. Representative photomicrographs showing the germinated spores and young gametophytes are presented in FIGURE 3.8-1. Inset A shows spores after 24 hours at 16.7 C, 110 $\mu E/m^2/s$ that have germinated and the spore contents have moved into the germination tube (arrows). The remaining insets provide a comparison of several treatments after 7 days. Inset B (16.7 C, 44 $\mu E/m^2/s$) can be compared with A to demonstrate the rate of development that occurred in the 6 days interval. Inset C (20.7 C, 110 $\mu E/m^2/s$) shows the arrested development that occurred at high temperatures. Inset D (ambient = 12.9 C, 77 $\mu E/m^2/s$)

TABLE 3.8-1

MATRIX OF TEMPERATURE AND LIGHT COMBINATIONS
TESTED IN EXPERIMENT 9B: TREE KELP
OPTIMUM TEMPERATURE FOR EARLY DEVELOPMENT

Light Intensity ($\mu E/m^2/s$)	Mean Temperature (C)					
	8.9	12.9 (ambient)	13.0	16.7	20.7	24.8
(dark)	X	X	X	X	X	X
44	X		X	X	X	X
77	X	X	X	X	X	X
110	X		X	X	X	X

"X" denotes a condition that was tested in this experiment.



See text for explanation.
Scale bar = 40 μm.

FIGURE 3.8-1

PTERYGOPHORA CALIFORNICA
OPTIMUM TEMPERATURE FOR EARLY DEVELOPMENT:
PHOTOMICROGRAPHS OF STAGES FROM SEVERAL TREATMENTS

shows the normal development of male (M) and female (F) gametophytes and inset E shows somewhat retarded development at 8.9 C, 110 $\mu E/m^2/s$.

Temperature Data

The temperatures given in TABLE 3.8-2 are the average temperatures in the dishes for the entire 168 hours of the experiment and the maximum and minimum temperatures recorded for each dish. The problem with day and night temperatures as affected by heating from artificial lights accounts for the fluctuating temperatures. The unusually low minima for a few treatments are explained by the fact that the probes were placed in those dishes closer to the time of the computer record than for the other dishes. All further references to temperature are the mean values of the four temperature records for each condition as given in TABLE 3.8-2.

Germination

For the initial germination counts after the 6-hour spore settlement period, no germination was observed (TABLE 3.8-3). Germination at the 24-hour sampling was high in all conditions except all light treatments at 24.8 C and the 16.7 C, 110 $\mu E/m^2/s$ treatment. The low point at 16.7 C was caused by a large patch of ungerminated spores on the coverslip. This condition was not observed again when the culture was sampled at 72 hours. Germination at 24.8 C increased slightly at the 48-hour sampling for the two lower light levels but decreased at the higher light levels. Bacterial contamination was heavy in these cultures and probably caused deterioration of some of the spores. Germination failed to reach 95 percent in only two cases: the dark and 44 $\mu E/m^2/s$ cultures at 13 C. In both cases, patches of ungerminated spores were encountered during sampling. Light intensity appeared to have no effect on germination.

Growth

Since spores at 24.8 C had not developed filaments after 72 hours, there are no growth data for that temperature. The average lengths of 25 gametophytes from

TABLE 3.8-2

SUMMARY TEMPERATURE DATA FOR EXPERIMENT 9B:
TREE KELP OPTIMUM TEMPERATURE FOR EARLY DEVELOPMENT

Target Temperature (C)	Target Light Intensity ($\mu E/m^2/s$)	Actual Temperature (C) $\bar{X}$	SD	Minimum	Maximum
25	77	24.8	0.7	18.8	25.4
	110	24.9	0.7	19.5	25.4
	44	24.7	0.6	20.6	25.3
	dark	24.6	0.5	20.2	25.2
21	77	20.7	0.7	16.8	21.5
	110	20.8	0.8	15.4	21.7
	44	20.7	0.6	19.4	21.5
	dark	20.6	0.6	19.3	21.4
17	77	16.7	0.6	15.5	17.4
	110	16.7	0.6	15.4	17.5
	44	16.7	0.6	15.5	17.5
	dark	16.7	0.6	15.5	17.4
13	77	13.0	0.5	11.8	13.6
	110	13.0	0.5	11.8	13.7
	44	13.0	0.5	11.8	13.6
	dark	13.0	0.6	11.9	14.1
9	77	9.1	0.3	8.2	9.5
	110	8.8	0.3	8.0	9.2
	44	8.7	0.2	8.0	9.0
	dark	9.1	0.3	8.3	9.5
ambient	dark	12.9	0.3	12.4	13.8

TABLE 3.8-3

MEAN PERCENTAGE GERMINATION OF ZOOSPORES:
TREE KELP OPTIMUM TEMPERATURE FOR EARLY DEVELOPMENT

Sample Time (H)	Light Intensity ($\mu E/m^2/s$)	Treatment Temperature 8.9	12.9	13.0	16.7	20.7	24.8
24	110	96.5	–	93.4	27.3	90.4	4.7
	77	89.0	97.7	99.3	88.9	98.3	8.3
	44	97.7	–	89.9	94.0	93.3	1.1
	dark	96.3	93.1	84.3	97.6	98.3	0.4
48	110	a	–	98.9	x	97.6	0.7
	77	x	a	a	98.6	98.2	0.6
	44	a	–	93.3	98.9	95.0	1.6
	dark	95.8	98.5	92.5	a	97.2	1.7
72	110	a	–	a	98.1	a	x
	77	97.2	a	a	a	a	x
	44	a	–	x	a	a	x
	dark	a	a	89.0	a	a	x

a – Germination previously recorded at or exceeding 95%, not sampled

x – Not sampled due to coverslip loss (except at 24.8 C)

the remaining conditions are presented in TABLE 3.8-4 and are plotted on a three-dimensional graph in FIGURE 3.8-2. After three days, mean lengths did not differ much between most of the culture conditions. Exceptions occur at 20.7 C dark, 13 C 110 $\mu E/m^2/s$ and 8.9 C 110 and 77 $\mu E/m^2/s$, where mean values were smaller than for the other treatments. More pronounced differences in size were obvious after seven days. The plants at 8.9 and 20.7 C and those under dark conditions showed very little growth. The longest filaments were at 16.7 C, in all light conditions. There appears to be very little effect of light intensity within any one temperature.

In contrast to bull kelp filaments, which were morphologically identical after seven days, filaments of tree kelp in light conditions at ambient, 13 and 16.7 C displayed two distinct morphological types. One type was a drop-shaped cell on the germination tube and was assumed to be a female gametophyte (see FIGURE 3.8-1). The other type was a slender filament, having either branches or small lateral cells, and was assumed to be a male gametophyte. These two morphologies were not observed at 8.9 and 20.7 C, further indicating a lack of development at these temperatures.

3.8.1.3 DISCUSSION

Germination of tree kelp zoospores was almost completely inhibited at 24.8 C (76.6 F). At the other temperatures tested, the germination rates were relatively uniform and appeared to be unaffected by light intensity. In contrast to germination, growth at both 8.9 and 20.7 C (48.0 and 69.3 F) was minimal. Absence of the two morphological types also indicated a lack of development at these temperatures. Growth increased from 8.9 to 16.7 C (48.0 to 62.1 F) and dropped off between 16.7 and 20.7 C (62.1 and 69.3 F).

The difference in growth between the 77 $\mu E/m^2/s$ cultures at ambient (12.9 C = 55.2 F) and 13 C (55.4 F) was due to the longer light period to which the ambient culture was exposed. Within each temperature, light intensity had very little effect on growth, indicating that light saturation may occur at or below 44 $\mu E/m^2/s$.

TABLE 3.8-4

MEAN LENGTHS OF GAMETOPHYTE FILAMENTS:
TREE KELP OPTIMUM TEMPERATURE FOR EARLY DEVELOPMENT

Sample Period (d)	Temperature (C)	Light Intensity ($\mu E/m^2/s$) dark	44	77	110
3	8.9	31.8 ± 3.6*	25.1 ± 5.4	22.2 ± 2.0	20.2 ± 6.0
	ambient (12.9)	24.3 ± 7.5	–	26.0 ± 3.8	–
	13.0	29.1 ± 4.8	26.5 ± 4.8	26.8 ± 8.2	21.5 ± 4.4
	16.7	26.3 ± 4.5	28.8 ± 7.5	27.3 ± 8.0	27.3 ± 3.5
	20.7	19.8 ± 5.3	25.6 ± 3.8	24.8 ± 5.7	23.5 ± 4.3
7	8.9	31.4 ± 4.4	27.2 ± 6.2	29.8 ± 4.8	23.8 ± 5.0
	ambient (12.9)	27.4 ± 5.4	–	34.5 ± 6.8	–
	13.0	31.3 ± 5.4	32.3 ± 4.7	3.05 ± 7.0	31.8 ± 4.6
	16.7	24.9 ± 4.9	37.8 ± 8.7	38.9 ± 6.5	38.1 ± 8.9
	20.7	20.6 ± 2.2	26.8 ± 8.1	26.5 ± 8.7	26.0 ± 5.5

* Length units are $\mu M \pm$ standard deviation (n = 25).

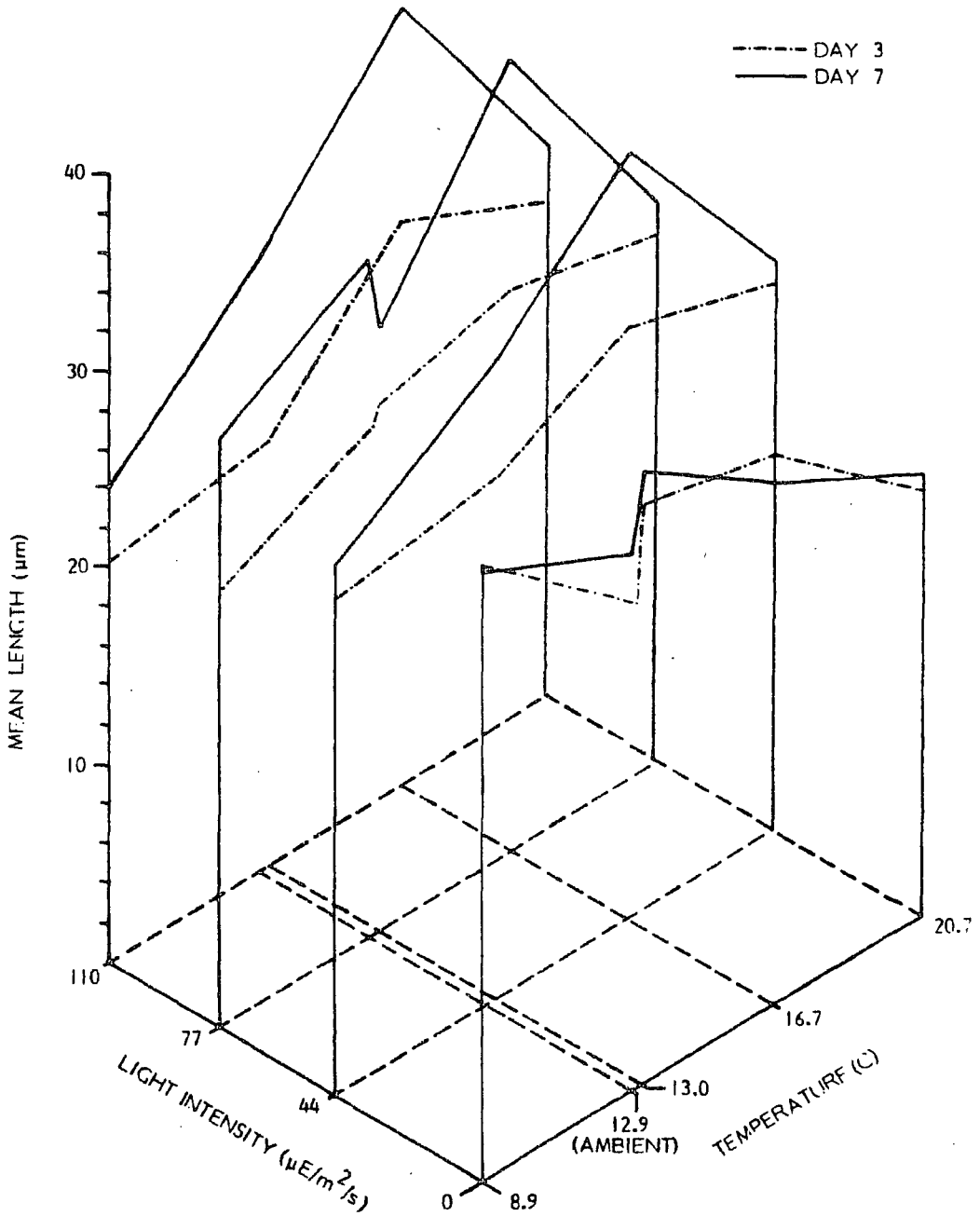


FIGURE 3.8-2

MEAN FILAMENT LENGTH VERSUS TEMPERATURE AND LIGHT INTENSITY AT DAYS 3 AND 7: TREE KELP GAMETOPHYTE OPTIMUM TEMPERATURE FOR GROWTH

No growth was observed at 24.8 C.

The growth data for both temperature and light compare very well to growth data collected for various Laminariales species (including Pterygophora) from Santa Barbara (LUnning and Neushul 1978). In their study, growth of female gametophytes peaked at 17 C (62.6 F) and was light saturated between 40 to 60 $\mu E/m^2/s$.

Optimum germination of zoospores takes place over the range of 8.9-20.7 C (48.0-69.3 F). At 24.8 C (76.6 F) germination is very low (generally less than 5 percent). A biologically meaningful maximum temperature for germination could be taken as the 50 percent germination level, which would be between 20.7 and 24.8 C (69.3 and 76.6 F). Further development into filaments was more sensitive to temperature than germination. Filament growth was optimal at 16.7 C (62.1 F) with the true optimum occurring in the range 13.0-20.7 C (55.4-69.3 F). With time, growth was markedly retarded at 8.9 and 20.7 C (48.0 and 69.3 F).

Early development of tree kelp gametophytes would be prevented at and above 24.8 C (76.6 F) and would be markedly arrested at 20.7 C (69.3 F). Decreasing temperatures would result in increasing rates of development down to approximately 16.7 C (62.1 F) where optimal development occurs.

3.8.2 GAMETOPHYTE GROWTH AND FERTILITY

The experiment described in this section follows continued growth and fertility of established tree kelp gametophytes and initial sporophyte production. The purpose of the experiment was to determine the thermal tolerance of tree kelp gametophytes. The objective was to define the optimum temperature for growth and initial sporophyte development under several light intensities.

3.8.2.1 METHODS

A brief summary of the methods used in this experiment is presented in this section. For a more detailed account see APPENDIX B (Procedure 318) and APPENDIX C (protocol, experiment 18b).

Fertile plants were collected in an area south of the power plant intake, using scuba equipment, on 13 November 1979 and transported to the lab in a damp cloth bag. They were held in running ambient seawater until spore release was induced.

Zoospores were seeded onto coverslips and cultured for one week in enriched seawater at 13 C. Light intensity was approximately 25 $\mu E/m^2/s$ with a 16-hour daylength. After one week, the coverslips were transferred to fresh enriched seawater and placed on the algal gradient tables at the Thermal Effects Laboratory at target temperatures of 9, 13, 17, 19, 20 and 23 C. This was day zero for the experiment. There were four separate cultures for each temperature: at light intensities of 143, 110, 77 and 44 $\mu E/m^2/s$, respectively.

Cultures were sampled every other day following day zero. Growth was assessed only on days 2, 4 and 6 by measuring 25 gametophytes per coverslip. Cultures were observed for fertility during all samplings. Once fertility was observed, the percentage fertile males and females of 50 randomly chosen gametophytes was recorded. For fertile females, the number of eggs and/or sporophytes per female was recorded.

3.8.2.2 RESULTS

A summary of the temperature data and the growth and fertility data are presented in this section.

Temperature Data

The mean temperature and standard deviation recorded for each probe for the entire experiment are presented in TABLE 3.8-5, along with maximum and minimum values, which represent the differences between daylight (added heat from fluorescent lights) and night conditions. The mean value of the four mean temperatures for each light level is also shown (combined mean) and is used in the following text in describing results.

TABLE 3.8-5

SUMMARY OF TEMPERATURE DATA:
TREE KELP GAMETOPHYTE OPTIMUM TEMPERATURE FOR GROWTH AND FERTILITY

Target Temperature (C)	Target Light Intensity ($\mu E/m^2/s$)	Actual Temperature (C)				
		Mean	Standard Deviation	Maximum	Minimum	Combined Mean
9	143	9.1	0.18	9.6	8.8	9.2
	110	9.2	0.19	9.8	8.9	
	77	9.1	0.15	9.6	8.8	
	44	9.2	0.19	9.6	8.8	
13	143	12.1	0.54	12.9	11.1	12.3
	110	12.2	0.52	13.1	11.2	
	77	12.3	0.52	13.2	11.3	
	44	12.5	0.50	13.2	11.5	
17	143	17.6	0.80	19.1	16.2	17.4
	110	17.4	0.68	18.4	16.2	
	77	17.3	0.67	18.2	16.2	
	44	17.3	0.67	18.2	16.2	
19	143	18.1	0.18	18.8	16.9	18.1
	110	18.2	0.19	19.0	17.0	
	77	18.1	0.15	18.9	17.0	
	44	18.0	0.19	18.7	16.9	
20	143	19.1	0.76	20.1	17.8	19.1
	110	19.1	0.77	20.1	17.8	
	77	19.2	0.76	20.1	17.8	
	44	19.0	0.75	20.0	17.7	
23	143	22.6	0.80	23.6	21.2	22.6
	110	22.7	0.68	23.6	21.3	
	77	22.6	0.67	23.4	20.9	
	44	22.4	0.67	23.2	20.6	

Growth

Mean lengths of 30 gametophytes for each light and temperature combination at days 2, 4 and 6 are shown in TABLE 3.8-6 and are plotted on three dimensional graphs in FIGURES 3.8-3, 3.8-4, and 3.8-5. At day 2, effects of light intensity and temperature were negligible (FIGURE 3.8-3). Differences between treatments became more pronounced with time (FIGURES 3.8-4 and 3.8-5). A two-way analysis of variance performed on mean lengths at day 6 showed significant effects from light, temperature and the interaction of these two factors (TABLE 3.8-7). Individual means were compared using the least significant difference (LSD) test, the results of which are shown in FIGURE 3.8-6.

Plants in all light intensities at 22.6 C failed to develop (see TABLE 3.8-6); therefore, those mean lengths are significantly smaller than all other means. Plants at 9.2 and 12.3 C (with the exception of 143 $\mu E/m^2/s$ at 12.3 C) formed the next major size group and were significantly smaller than all the 17.4, 18.1 and 19.1 C plants. Peak growth occurred in three light intensities at 18.1 C (44, 77 and 10 $\mu E/m^2/s$) and in 77 $\mu E/m^2/s$ at 17.4 and 19.1 C. There were few significant light intensity effects within temperatures, the most notable ones being a significantly higher mean at 44 $\mu E/m^2/s$ in 12.3 C plants and a significantly lower mean at 143 $\mu E/m^2/s$ in 18.1 C plants.

Fertility

The percentage fertile male and female plants along with the mean number of eggs per female are presented in TABLE 3.8-8. At day 8, male fertility was generally high (greater than 80 percent) in the three highest light levels at all temperatures except 19.1 C. The lowest fertility rates (0 and 1 percent) were observed at the lowest light levels in 12.3 and 18.1 C. By day 12, male fertility exceeded 90 percent in all cases except 44 $\mu E/m^2/s$ plants at 17.4 and 19.1 C (FIGURES 3.8-7, 3.8-8, and 3.8-9). In these two conditions male fertility actually decreased from day 8. There is no obvious explanation for this decrease.

TABLE 3.8-6

MEAN FILAMENT LENGTH (STANDARD DEVIATIONS IN PARENTHESES):
TREE KELP GAMETOPHYTE OPTIMUM TEMPERATURE FOR GROWTH AND FERTILITY

Sample Time (days)	Light Intensity ($\mu E/m^2/s$)	Mean Temperature (C)											
		9.2		12.3		17.4		18.1		19.1		22.6	
2	143	42.5	(5.6)	46.0	(7.2)	49.1	(5.4)	45.8	(8.9)	48.6	(5.9)	43.2	(7.3)
	110	40.0	(5.8)	45.5	(7.9)	50.8	(6.2)	51.9	(9.0)	48.8	(6.5)	40.0	(4.5)
	77	47.2	(6.3)	44.3	(7.4)	46.9	(5.9)	51.6	(8.2)	44.0	(7.5)	38.7	(4.0)
	44	41.5	(4.7)	45.7	(7.6)	44.2	(5.8)	50.4	(8.1)	49.5	(6.8)	39.5	(4.2)
4	143	62.2	(11.9)	61.6	(10.3)	68.7	(10.3)	74.9	(9.8)	71.3	(10.4)	37.7	(4.5)
	110	54.6	(10.2)	60.4	(11.2)	82.4	(9.4)	81.7	(12.0)	74.7	(9.8)	38.5	(5.0)
	77	53.0	(7.0)	68.7	(12.3)	70.7	(10.5)	82.0	(11.8)	70.5	(13.5)	42.7	(6.4)
	44	38.8	(4.3)	64.0	(6.8)	68.7	(9.9)	95.2	(13.1)	78.4	(13.7)	39.5	(4.8)
6	143	65.3	(11.3)	71.6	(16.1)	96.1	(15.4)	88.9	(17.6)	92.7	(13.4)	40.0	(5.0)
	110	66.0	(7.8)	74.6	(13.4)	97.6	(18.3)	101.0	(17.5)	88.9	(14.2)	36.3	(5.3)
	77	63.2	(9.3)	78.2	(13.5)	102.5	(19.5)	107.1	(19.8)	99.6	(18.3)	39.2	(4.3)
	44	60.6	(9.9)	94.9	(20.1)	91.5	(12.8)	107.1	(19.0)	96.2	(14.8)	37.8	5.8)

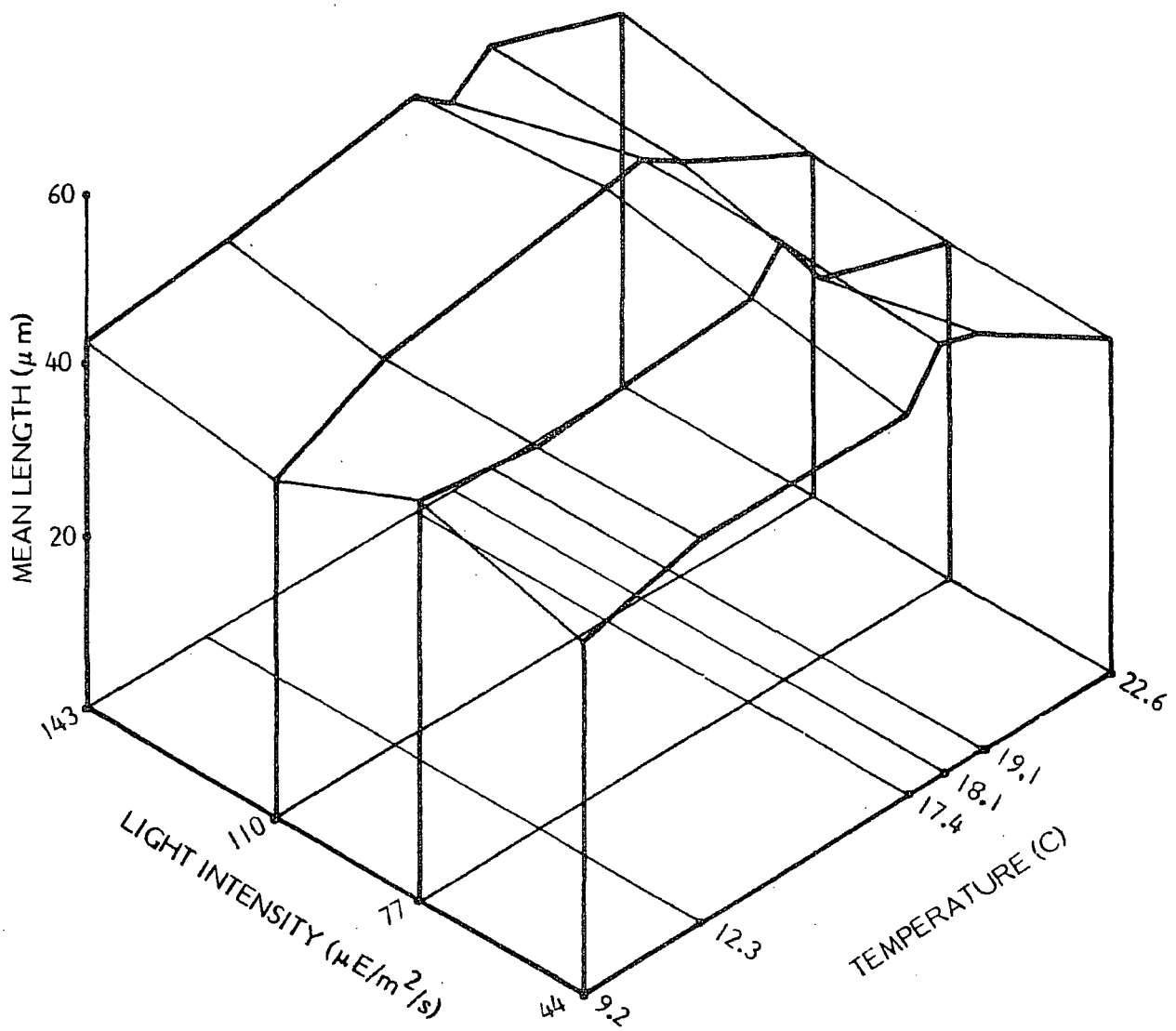


FIGURE 3.8-3

MEAN FILAMENT LENGTH VERSUS TEMPERATURE AND LIGHT INTENSITY AT DAY 2: TREE KELP GAMETOPHYTE OPTIMUM TEMPERATURE FOR GROWTH AND FERTILITY

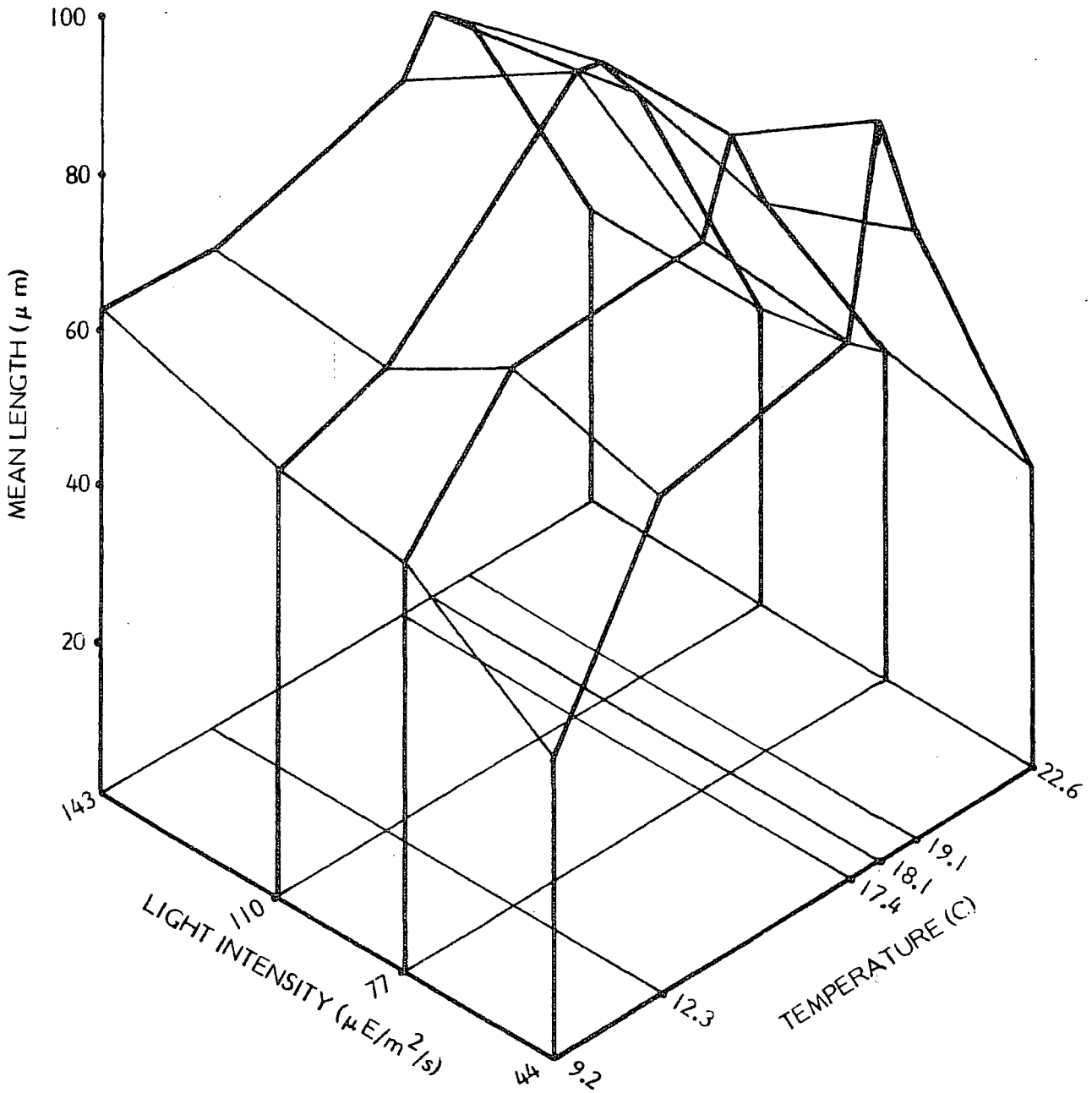


FIGURE 3.8-4

MEAN FILAMENT LENGTH VERSUS TEMPERATURE AND LIGHT INTENSITY AT DAY 4: TREE KELP GAMETOPHYTE OPTIMUM TEMPERATURE FOR GROWTH AND FERTILITY

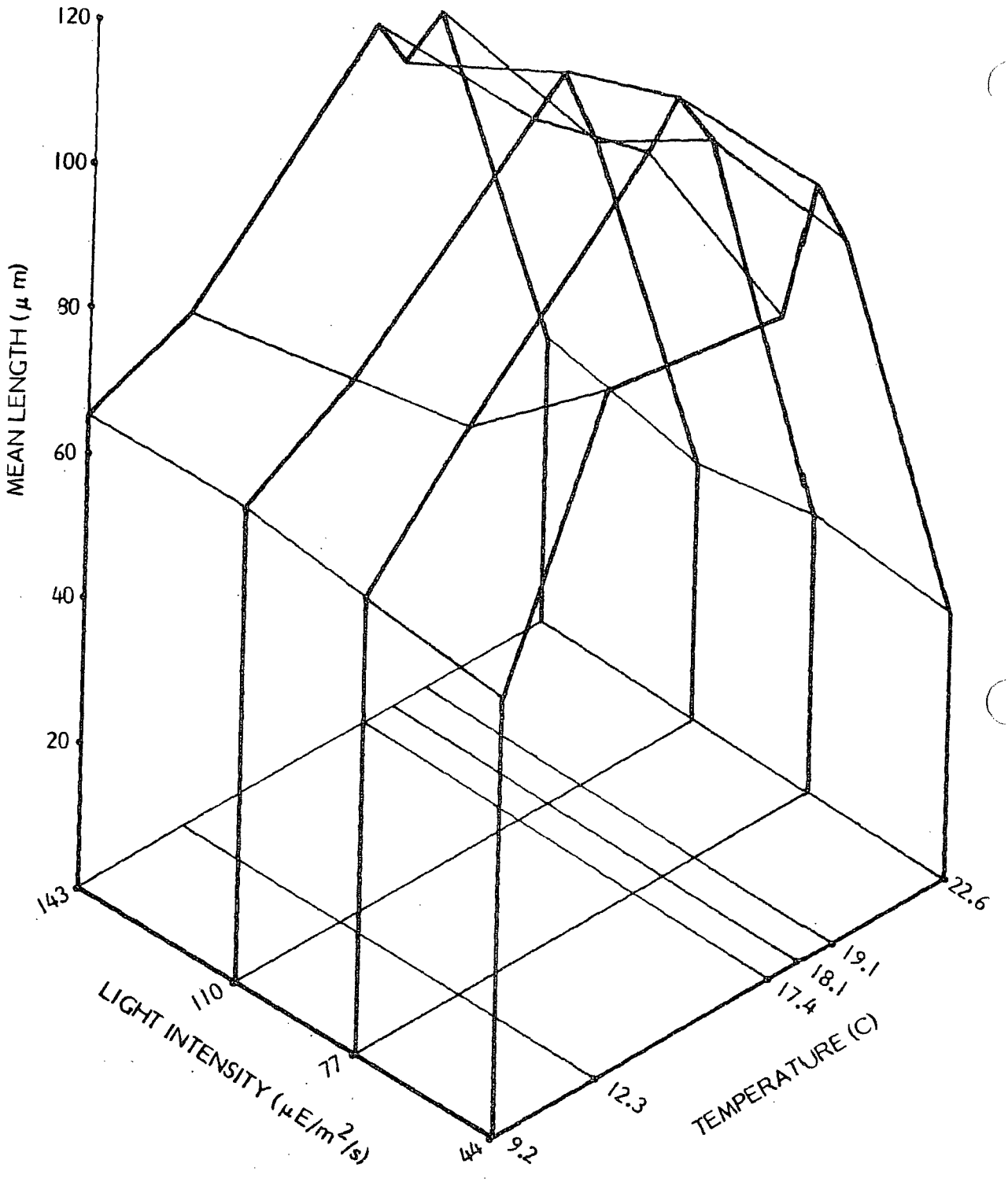


FIGURE 3.8-5

MEAN FILAMENT LENGTH VERSUS TEMPERATURE AND LIGHT INTENSITY AT DAY 6: TREE KELP GAMETOPHYTE OPTIMUM TEMPERATURE FOR GROWTH AND FERTILITY

TABLE 3.8-7

2-WAY ANALYSIS OF VARIANCE ON MEAN LENGTH AT DAY 6:
TREE KELP GAMETOPHYTE OPTIMUM TEMPERATURE FOR GROWTH AND FERTILITY

Light Intensity ($\mu E/m^2/s$)	Temperature (C) 9.2	12.3	17.4	18.1	19.1	22.6	
143	1,959	2,148	2,883	2,667	2,781	1,200	13,638
110	1,980	2,238	2,928	3,030	2,667	1,089	13,932
77	1,896	2,346	3,075	3,213	2,988	1,176	14,694
44	1,818	2,847	2,745	3,213	2,886	1,134	14,643
	7,653	9,579	11,631	12,123	11,322	4,599	56,907

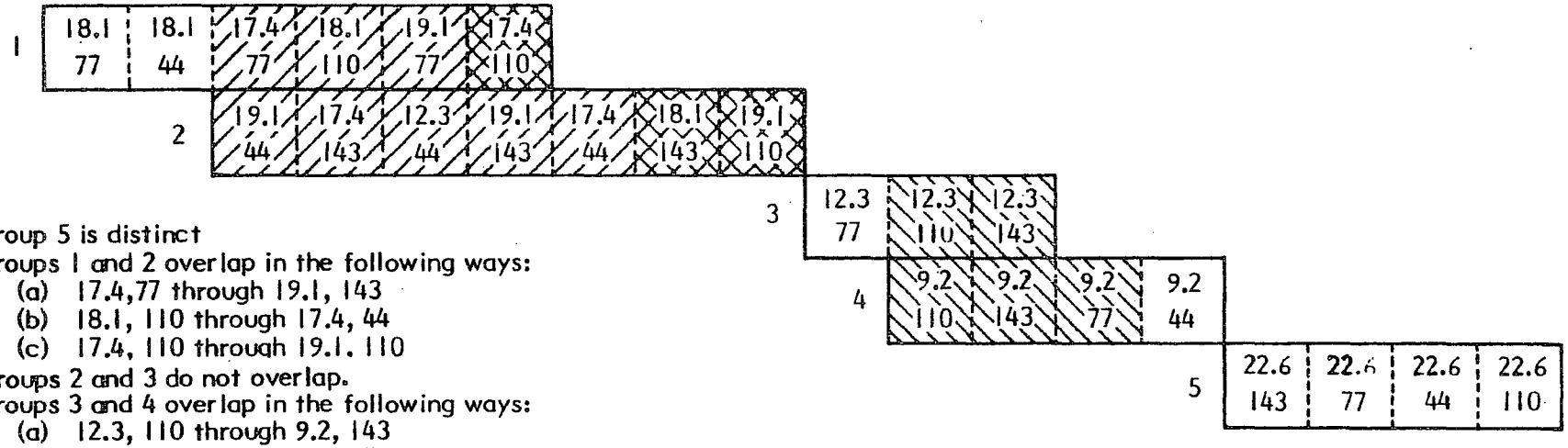
Numbers in cells are $\sum x$'s, n = 30. $\sum x^2$ = 5,019,604.69

ANOVA

Source	df	SS	MS	Fcomp	F.99
Subgroups	23	372,365.89			
Light	3	4,583.84	1,527.95	7.12	3.78
Temperature	5	351,474.86	70,294.97	327.36	3.02
Interaction	15	16,307.19	1,087.15	5.06	2.04
Error	696	149,451.79	214.73		
Total	719	521,817.68			

$$LSD = t_{.01/2\,\infty} \sqrt{\frac{2}{30} 214.73} = 9.75$$

Numbers in squares are temperature (C) values (top) and light ($\mu E/m^2/s$) values (bottom). Mean length values decrease from left to right and from top to bottom. Members of groups are statistically the same (p = .01).



Group 5 is distinct
Groups 1 and 2 overlap in the following ways:
(a) 17.4,77 through 19.1, 143
(b) 18.1, 110 through 17.4, 44
(c) 17.4, 110 through 19.1. 110
Groups 2 and 3 do not overlap.
Groups 3 and 4 overlap in the following ways:
(a) 12.3, 110 through 9.2, 143
(b) 12.3, 143 through 9.2, 77

FIGURE 3.8-6

RESULTS OF LSD TEST ON MEAN LENGTHS AT DAY 6: TREE KELP GAMETOPHYTE OPTIMUM TEMPERATURE FOR GROWTH AND FERTILITY

TABLE 3.8-8

FERTILITY AT DAYS 8, 10 AND 12:
TREE KELP GAMETOPHYTE OPTIMUM TEMPERATURE
FOR GROWTH AND FERTILITY

Temperature (C)	Light Intensity ($\mu Em^{-2}s^{-1}$)	Day 8			Day 10			Day 12		
		Female % Fertile	$\bar{X}$ No. Eggs[a]	Male % Fertile	Female % Fertile	$\bar{X}$ No. Eggs[a]	Male % Fertile	Female % Fertile	$\bar{X}$ No. Eggs[a]	Male % Fertile
9.2	143	60*	1.0 (0.2)	98	42*	1.0 (0.0)	100	86*	1.2 (0.4)	100
9.2	110	44*	1.0 (0.2)	86	48*	1.1 (0.3)	98	88*	1.3 (0.4)	100
9.2	77	0**	—	16	16*	1.0 (0.0)	100	22*	1.0 (0.0)	100
9.2	44	74*	1.0 (0.0)	96	48*	1.0 (0.0)	100	48*	1.0 (0.0)	100
12.3	143	74*	1.3 (0.5)	100	88*	1.3 (0.5)	100	96*	1.7 (0.8)	100
12.3	110	58*	1.1 (0.4)	100	94*	1.4 (0.6)	100	96*	1.6 (0.7)	100
12.3	77	60*	1.2 (0.4)	98	92*	1.3 (0.5)	100	100*	1.9 (0.9)	100
12.3	44	16*	1.0 (0.0)	0	70*	1.0 (0.2)	92	100*	1.3 (0.4)	100
17.4	143	44	1.2 (0.4)	100	74	1.8 (0.8)	100	84*	2.0 (0.9)	100
17.4	110	40	1.2 (0.4)	98	68	1.7 (0.8)	100	90*	2.2 (0.9)	100
17.4	77	64	1.1 (0.3)	100	64	1.2 (0.4)	100	88*	1.6 (0.7)	98
17.4	44	8	1.0 (0.0)	58	0	—	08	36	1.0 (0.0)	38
18.1	143	62	2.0 (0.9)	98	100	2.9 (1.3)	100	96*	3.3 (1.1)	100
18.1	110	72	2.1 (0.9)	90	100	3.5 (1.3)	100	100*	3.5 (1.6)	100
18.1	77	64	2.1 (0.8)	86	92	2.0 (0.9)	100	98	3.3 (1.1)	100
18.1	44	16	1.0 (0.0)	01	14	1.1 (0.4)	38	66	1.5 (0.6)	94
19.1	143	2	1.0 (0.0)	70	6	1.0 (0.0)	76	24	1.1 (0.3)	100
19.1	110	14	1.1 (0.4)	68	18	1.3 (0.5)	92	36	1.2 (0.4)	98
19.1	77	10	1.0 (0.0)	74	8	1.0 (0.0)	80	24	1.4 (0.5)	98
19.1	44	14	1.0 (0.0)	84	6	1.0 (0.0)	52	10	1.6 (0.9)	08

* Most eggs extruded from oogonia.
** Fertile females were observed but were not hit by the random points.
a Standard deviation indicated in parentheses.

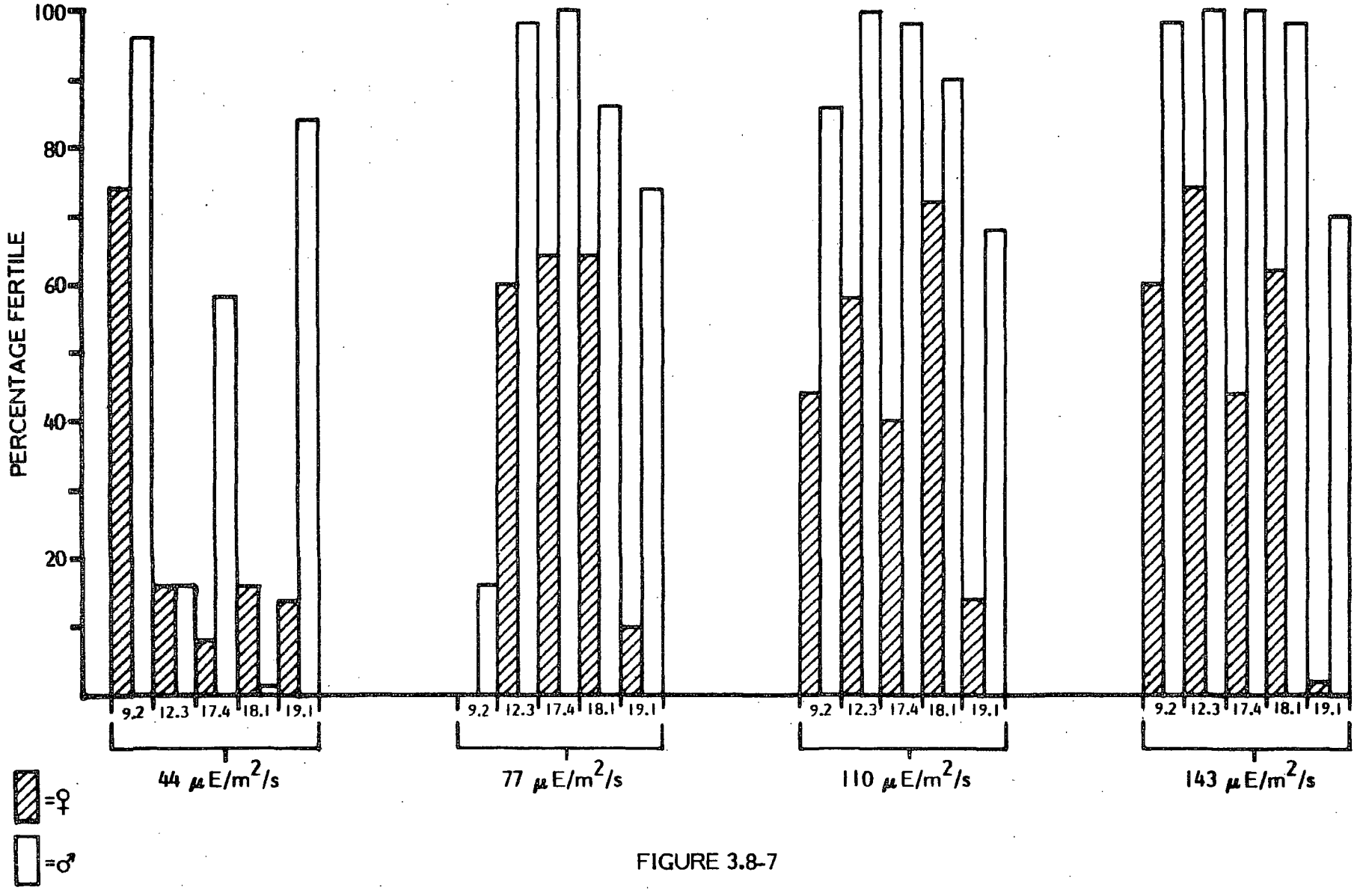


FIGURE 3.8-7

PERCENTAGE FERTILE MALE AND FEMALE PLANTS AT DAY 8:
TREE KELP GAMETOPHYTE OPTIMUM TEMPERATURE
FOR GROWTH AND FERTILITY

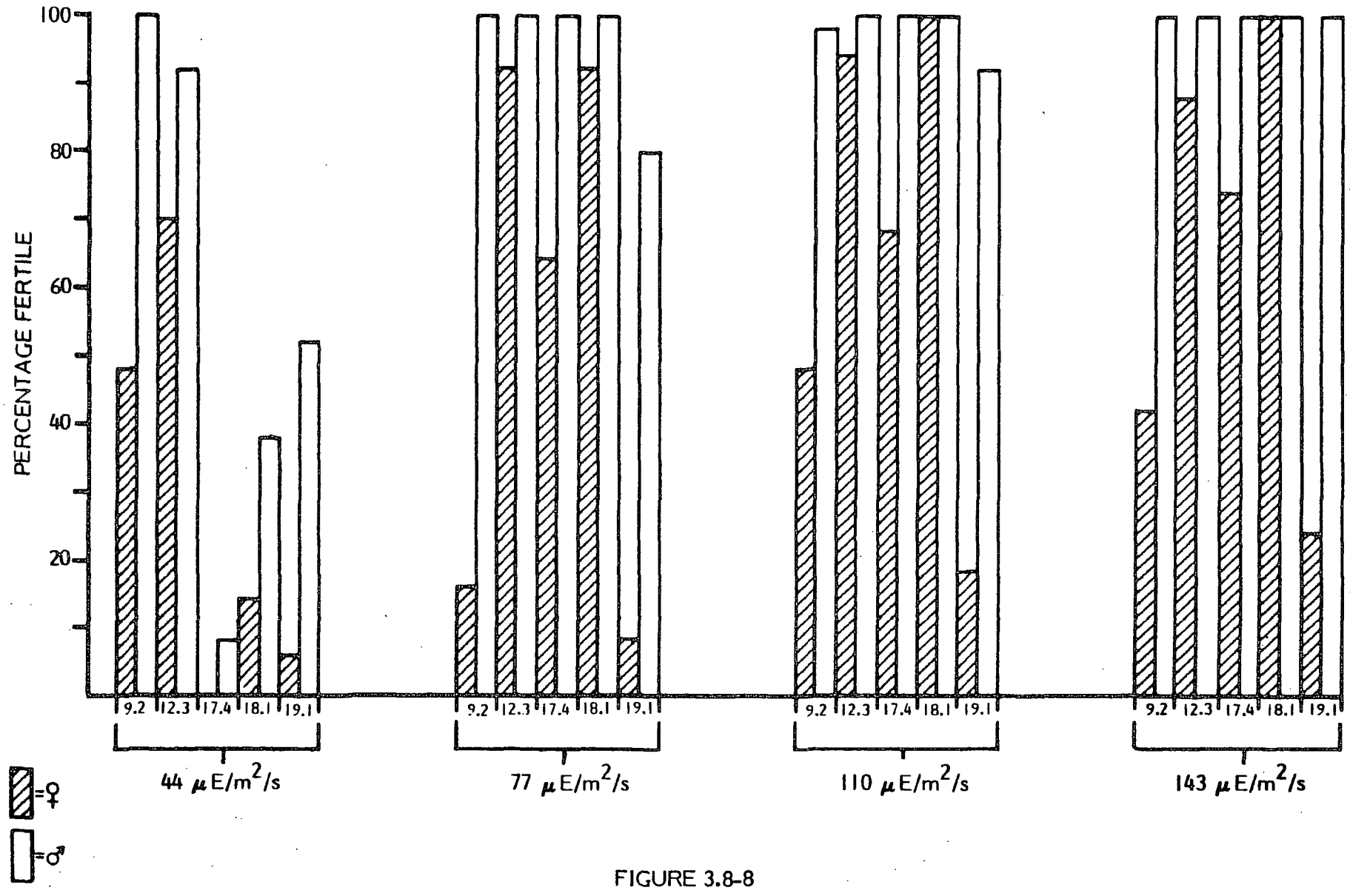


FIGURE 3.8-8

PERCENTAGE FERTILE MALE AND FEMALE PLANTS AT DAY 10:
TREE KELP GAMETOPHYTE OPTIMUM TEMPERATURE
FOR GROWTH AND FERTILITY

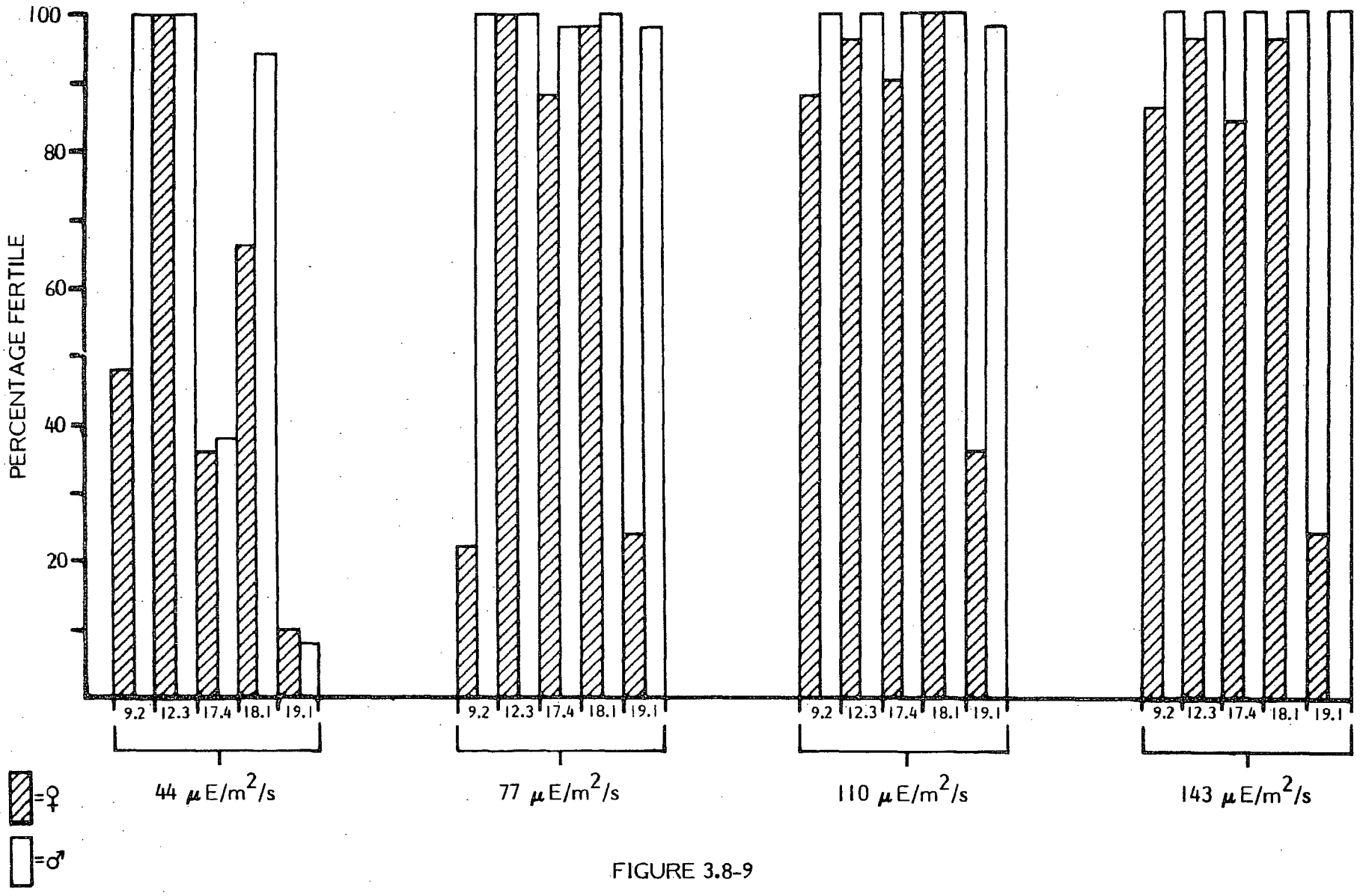


FIGURE 3.8-9

PERCENTAGE FERTILE MALE AND FEMALE PLANTS AT DAY 12:
TREE KELP GAMETOPHYTE OPTIMUM TEMPERATURE
FOR GROWTH AND FERTILITY

Female fertility at day 8 followed the same general pattern as male fertility, with the lowest values at 44 $\mu E/m^2/s$ (9.2 C is an exception). At day 12 reduced values at 44 $\mu E/m^2/s$ were still evident, except at 12.3 C. Fertility for the most part was not affected at the other light intensities (TABLE 3.8-9).

The most striking trend in female fertility at day 12 was the decrease at 19.1 C, which is significant for all light levels (TABLE 3.8-10). Other temperature effects are significant but only at certain light levels (see TABLE 3.8-10).

Gametophytes at 9.2 and 12.3 C matured faster than gametophytes at the other temperatures. At day 8, both extruded eggs and sporophytes were observed in 9.2 and 12.3 C samples. Neither one of these traits was observed in the other temperatures at that time (TABLES 3.8-10 and 3.8-11). By day 12, extruded eggs were also observed in the higher light intensity cultures at 17.4 and 18.1 C. Sporophytes were observed in limited numbers only at 17 C, 77 $\mu E/m^2/s$ and 18.1 C, 110 $\mu E/m^2s$. Lack of extruded eggs and very few sporophytes at 19.1 C further emphasize reduced development at this temperature.

3.8.2.3 DISCUSSION

Vegetative growth of tree kelp gametophytes reaches an optimum in a range of temperatures from 17.4 to 19.1 C (63.3 to 66.4 F) and was completely inhibited at 22.6 C (72.7 F). At all of the temperatures tested, light intensity effects were minimal, indicating saturation around 44 $\mu E/m^2/s$. These results agree with a previous experiment on tree kelp (see previous section) and with results obtained by LÜnning and Neushul (1978) for tree kelp (peak female growth at 17 C (63 F), light saturated at 40-60 $\mu E/m^2/s$.

Although vegetative growth was significantly lower at 12.3 and 9.2 C (54.1 and 48.6 F), peak female fertility (in terms of sporophyte production) occurred at these temperatures. Fertile females were observed at 17.4, 18.1 and 19.1 C (63.3, 64.6 and 66.4 F); however, within the time limit of this experiment, very few sporophytes were observed in these cultures. An increase in sporophyte numbers with time seems probable especially at 17.4 and 18.1 C (63.3 and

TABLE 3.8-9

CHI-SQUARE CONTINGENCY TABLES COMPARING FEMALE FERTILITY WITH LIGHT INTENSITY AT DAY 12: TREE KELP OPTIMUM TEMPERATURE FOR GROWTH AND FERTILITY

Temperature (C)		Light Intensity ($\mu E/m^2/s$) 143	110	77	44	$\sum$
9.2	Fertile	43	44	11	24	122
	Not Fertile	7	6	39	26	78
	$\sum$	50	50	50	50	200
	χ^2 = 63.97* (143 = 110, both larger than 77 and 44)					
12.3	Chi-square test was not possible at this temperature.					
17.4	Fertile	42	45	44	18	149
	Not Fertile	8	5	6	32	51
	$\sum$	50	50	50	50	200
	χ^2 = 52.51* (143, 110, 77 all = and larger than 44)					
18.1	Fertile	48	50	49	33	180
	Not Fertile	2	0	1	17	20
	$\sum$	50	50	50	50	200
	χ^2 = 43.11* (same as 17.4 C)					
19.1	χ^2 = 9.43 Light effect is not significant.					

* Significant at p = .01 (χ^2 .01 3df = 11.34)

TABLE 3.8-10

CHI-SQUARE CONTINGENCY TABLES COMPARING FEMALE FERTILITY WITH TEMPERATURE ON DAY 12: TREE KELP OPTIMUM TEMPERATURE FOR GROWTH AND FERTILITY

Light Intensity ($\mu E/m^2/s$)		Temperature (C) 9.2	12.3	17.4	18.1	19.1	$\sum$
143	Fertile	43	48	42	48	12	193
	Not Fertile	7	2	8	2	38	57
	$\sum$	50	50	50	50	50	250

χ^2 = 103.99* (19.1 lower than all others, which are equal)

110	Fertile	44	48	45	50	18	205
	Not Fertile	6	2	5	0	32	45
	$\sum$	50	50	50	50	50	250

χ^2 = 92.68* (19.1 lower than all others; 18.1 and 12.3 not different; 9.2, 17.4, 12.3 not different)

77	Fertile	11	50	44	49	12	166
	Not Fertile	39	0	6	1	38	84
	$\sum$	50	50	50	50	50	250

χ^2 = 142.61* (19.1 and 9.2 not different and both lower than all others)

44	Fertile	24	50	18	33	5	130
	Not Fertile	26	0	32	17	45	120
	$\sum$	50	50	50	50	50	250

χ^2 = 90.87* (19.1 lower than all others, 12.3 higher than all)

* Significant at p = .01 (χ^2 .01 4df = 13.28)

TABLE 3.8-11

SPOROPHYTE PRODUCTION AT DAYS 8, 10 AND 12: TREE KELP GAMETOPHYTE OPTIMUM TEMPERATURE FOR GROWTH AND FERTILITY

Temperature (C)	Light Intensity ($\mu Em^{-2}s^{-1}$)	Day 8			Day 10			Day 12		
		Total No. Females w/Sporophytes	Total No. Sporophytes	$\bar{X}$ No. Sporophytes/Female	Total No. Females w/Sporophytes	Total No. Sporophytes	$\bar{X}$ No. Sporophytes/Female	Total No. Females w/Sporophytes	Total No. Sporophytes	$\bar{X}$ No. Sporophytes/Female
9.2	143	2	2	1.0	8	8	1.0	22	24	1.1
9.2	110	—	—	—	4	4	1.0	10	10	1.0
9.2	77	—	—	—	1	1	1.0	1	1	1.0
9.2	44	8	8	1.0	14	14	1.0	18	18	1.0
12.3	143	2	2	1.0	14	14	1.0	10	10	1.0
12.3	110	8	8	1.0	18	18	1.0	11	11	1.0
12.3	77	—	—	—	3	3	1.0	19	19	1.0
12.3	44	2	2	1.0	3	3	1.0	30	33	1.1
17.4	143	—	—	—	—	—	—	—	—	—
17.4	110	—	—	—	—	—	—	—	—	—
17.4	77	—	—	—	—	—	—	1	1	1.0
17.4	44	—	—	—	—	—	—	—	—	—
18.1	143	—	—	—	—	—	—	—	—	—
18.1	110	—	—	—	—	—	—	3	3	1.0
18.1	77	—	—	—	—	—	—	—	—	—
18.1	44	—	—	—	—	—	—	—	—	—
19.1	143	—	—	—	—	—	—	—	—	—
19.1	110	—	—	—	—	—	—	—	—	—
19.1	77	—	—	—	—	—	—	—	—	—
19.1	44	—	—	—	1	1	1.0	—	—	—

64.6 F), where the mean number of eggs per female at day 12 was relatively high (TABLE 3.8-8). At 19.1 C (66.4 F), where female fertility was significantly lower, an increase in sporophyte production seems less likely. Adverse temperature effects have been observed for juvenile tree kelp sporophytes at 19.1 C (66.4 F) and a total inhibition of fertility was observed at 20 C (68 F) by Lüning and Neushul (1978).

Light saturation for female fertility varied with the different temperatures. At 9.2 C (48.6 F), saturation occurred around 110 $\mu E/m^2/s$ whereas at 17.4, 18.1 and 19.1 C (63.3, 64.6 and 66.4 F) saturation was near 77 $\mu E/m^2/s$. At 12.3 C (54.1 F), fertility was optimal in all light intensities, indicating saturation below 44 $\mu E/m^2/s$. Male fertility seemed to be relatively unaffected by either light or temperature.

3.8.3 SPOROPHYTE GROWTH

The objective of the tree kelp sporophyte growth experiment was elucidation of temperatures promoting maximum growth of the alga in the laboratory, for the purpose of more accurately predicting effects of the anticipated thermal discharge in Diablo Cove.

A preliminary tree kelp growth study was conducted from 30 November 1979 through 4 January 1980. Test temperatures, chosen with the aid of information gleaned from the tree kelp 96-hour LT50 experiment, were 12, 14, 16 and 18 C, as well as an ambient "control" treatment. Plants were maintained in the raceways using methods similar to those described for bull kelp sporophytes (see SECTION 3.7.3): i.e., clipping plants basally onto PVC racks situated in filled raceways of controlled water temperature. Weekly measurements were made, consisting of linear determination of increase or decrease in blade and stipe lengths.

Results indicated that temperatures of 18 C were at least marginally tolerated by the sporophytes. Additionally, plant growth at ambient temperatures was found not to be markedly different from that in other low temperature

treatments. Stipe growth was not detected during the relatively short experimental period (28 days).

It is important for the reader to note that tree kelp is a "pseudoperennial": that is, the holdfast and stipe overwinter while the blade and sporophylls are annually sloughed. These processes of blade degeneration and regeneration are strongly seasonal.

Introduction of tree kelp sporophytes into the laboratory has been found, in these experiments, to (artificially) induce extant blade degeneration and new blade initiation. Accurate linear measurement of blade length is precluded by these processes; rate of sloughing of the old blade can equal or exceed rate of formation of the new, thereby generating null or negative growth rate values where in fact ample growth may be occurring.

Another laboratory-induced characteristic is the tendency for blades generated in culture to be noticeably more expanded laterally than those produced in nature (this may possibly be attributed to decreased water motion in the raceways as compared with nature). For the above two reasons, linear measurements were found not to be descriptive of growth occurring among plants in the laboratory. Development of an appropriate means of surface area quantification was therefore undertaken and is described in the following subsection.

3.8.3.1 METHODS

Experimental methods are summarized below. For a more detailed presentation, refer to the detailed procedures in APPENDIX B (Procedure 322, Rev. 1) and protocol in APPENDIX C (experiment 22c).

Divers collected sporophytes from depths of 15 to 25 ft in Diablo Cove on 30 January 1980. Specimens collected were small, presumably second-season plants, without sporophylls.

In the laboratory, plants were held in raceways filled with flowing seawater at ambient temperature for a period of 48 h prior to experiment initiation. During this time, plants were carefully examined, and damaged or abnormal plants were discarded. The healthy plants were then sorted by total length so that the range of initial lengths would be minimized in the experiment. From this group, plants were haphazardly distributed among five experimental treatment groups of 25 individuals each. Each group comprised plants of blade surface areas roughly comparable to those of every other group. Plants were numbered for individual identification, and tied loosely with a length of teflon tape to stationary plastic clips.

Plants within each group were "temperature adjusted" to the designated treatment temperature by increasing or decreasing the water temperature at stepwise intervals of no more than 2 C in any eight hour period. This adjustment period was allowed in order to reduce the effects of thermal shock. The temperature adjustment period was scheduled so that day 0 of the experiment coincided with the day on which the tank reached the target temperature for that particular treatment.

Experiment treatment temperatures included 12, 14, 16, 18 and 20 C. No ambient seawater temperature treatment was employed. Treatments were maintained for five weeks, with the exception of the 20 C treatment, which was terminated after only three weeks due to inadequate temperature control.

Irradiance was provided by overhead banks of 25 W Duro Test Ultra-High Output fluorescent bulbs; a $16:\overline{8}$ (hours light:hours dark) photoregime was maintained throughout the experiment. Raceways and plants were cleaned of diatoms and other epiphytes periodically as described in the experiment procedure (see APPENDIX B).

Temperatures within each treatment were recorded by the Autodata Nine computer. A single YSI 401 temperature probe was positioned in each raceway. Light intensity (photon flux density) incident at the water surface was measured weekly during the experiment, using a Li-Cor Quantum Meter (Model LI-185) in

conjunction with a Li-Cor surface sensor. Water samples were taken weekly from each raceway and subsequently analyzed for pH, salinity, and ammonia, nitrite, nitrate, and phosphate ion concentrations.

At experiment initiation, and at weekly intervals thereafter, each plant was photographed against the white plexiglass background of a copy stand, using Kodak Panatomic-X film. Plants were oriented to expose the greatest amount of blade surface area to the camera. Bullate and corrugate blades, where possible, were flattened using coins as weights.

A 10 cm reference size scale was included in each photograph. Film strips were developed according to manufacturer's instructions. Strips were then viewed by means of a photographic enlarger, and tracings of blade and sporophyll perimeters were made for each plant at every measurement time. A Hewlett-Packard (HP) Digitizer (Model No. 9864A), in conjunction with a HP Keyboard (Model No. 9830A) and HP Printer (Model No. 9866A), was used to calculate surface area of blades and sporophylls from each tracing. Surface area quantifications were summarized as mean and standard deviation values for each treatment at each measurement time. Additionally, multiple regression analyses were performed and tested for significant differences between treatments.

3.8.3.2 RESULTS

Analyses of the temperature, light, water quality and growth data are presented in this section.

Temperature Data

Temperature data pertaining to the experiment are summarized in TABLE 3.8-12. Mean and standard deviation values represent compiled hourly data from the entire 35 day experimental period, except those for the 20 C treatment, which was terminated after 21 days. All treatments were subject to temperature variation throughout the experimental period. Greatest variation occurred in the 17.9 C treatment, which ranged 0.4 C above and 1.5 C below the

TABLE 3.8-12

STATISTICAL SUMMARY OF TEMPERATURE (C) DATA:
TREE KELP SPOROPHYTE OPTIMUM TEMPERATURE FOR GROWTH

Target Temperatures	Actual Temperatures			
	Mean	Standard Deviation	Maximum	Minimum
12	11.9	0.11	13.5	11.7
14	13.9	0.14	14.6	13.5
16	15.8	0.19	16.1	15.2
18	17.9	0.16	18.4	16.5
20	19.9	0.10	20.4	19.6

designated target temperature. The 11.9 C treatment showed a maximum variation of 1.8 C, with a maximum increase over target temperature of 1.5 C. The 13.9 C treatment varied 1.1 C, with a maximum increase of 0.6 C above target temperature. Within the 15.8 C treatment, a maximum increase of only 0.1 C above target temperature was experienced. The 19.9 C treatment ranged from 19.6 to 20.4 C.

Light Data

TABLE 3.8-13 shows maximum and minimum light intensity (photon flux density) values incident at the water surface of each raceway at each of four sample times. Each value represents an instantaneous, single point measurement made at one of four predesignated positions above each raceway. Intensities varied both within and between raceways at all measurement times. The highest value measured was 138 $\mu E/m^2/s$, and the lowest was 86 $\mu E/m^2/s$. The 11.9, 13.9 and 19.9 C treatments were exposed to intensities slightly, but consistently, less than the 15.8 C and 17.9 C treatments.

Water Quality Data

Results of water sample analyses are presented in TABLE 3.8-14. Given values represent concentrations measured in discrete samples collected from each treatment raceway at five sample times. Aliquots were assayed for pH and salinity, and for ammonia, nitrite, nitrate, and phosphate concentrations. pH values ranged from a low of 7.7 (1 February) to a high of 8.1 (28 February) but were nearly identical between raceways at a given measurement time, varying only between sample periods. Salinity remained at 32-33 ppt (parts per thousand) in all samples but one on 6 March which measured 34 ppt. Ammonia concentrations in all samples remained below levels of detectability at < 0.3 μ g/l. Nitrite concentrations were below levels of detectability in all samples collected on 7 and 28 February, and on 6 March. Samples from the two intermediate weeks of 14 and 21 February varied in nitrite concentration from a minimum of < 3.0 μg/l to a maximum of 13 μg/l.

TABLE 3.8-13

RANGES OF LIGHT INTENSITY VALUES
RECORDED DURING THE EXPERIMENT:
TREE KELP SPOROPHYTE OPTIMUM TEMPERATURE FOR GROWTH

Ranges in $\mu E/m^2/s$ from four sampling periods.

Date	Treatment Group									
	12 C		14 C		16 C		18 C		20 C	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
02/08/80	86	109	96	117	101	132	103	133	86	111
02/15/80	90	109	100	121	103	133	103	135	92	116
02/22/80	98	117	103	124	108	135	103	134	94	114
02/28/80	102	121	102	121	104	138	103	138	Terminated	

TABLE 3.8-14

RESULTS OF WATER QUALITY ANALYSES:
TREE KELP SPOROPHYTE OPTIMUM TEMPERATURE FOR GROWTH

Units are as follows: for NH_3 (ammonia), NO_2 (nitrite), NO_3 (nitrate), and PO_4 (phosphate) as $\mu g/l$; salinity as parts per thousand.

Sample Date	Target Temperature (C)	Parameter					
		pH	NH_3	NO_2	NO_3	PO_4	Salinity
7 February	12	7.7	<0.3	<3.0	<10	30	33
	14	7.7	<0.3	<3.0	<10	15	33
	16	7.7	<0.3	<3.0	<10	15	33
	18	7.7	<0.3	<3.0	<10	15	33
	20	7.7	<0.3	<3.0	<10	40	33
14 February	12	8.0	<0.3	<3.0	10	15	33
	14	8.0	<0.3	11.0	<10	15	32
	16	8.0	<0.3	<3.0	<10	15	32
	18	7.9	<0.3	13.0	<10	30	32
	20	7.9	<0.3	<3.0	<10	15	32
21 February	12	7.9	<0.3	11.0	<10	40	32
	14	7.9	<0.3	9.0	<10	30	32
	16	7.9	<0.3	6.0	<10	40	32
	18	7.9	<0.3	13.0	<10	30	32
	20	7.9	<0.3	9.0	<10	40	32
28 February	12	8.0	<0.3	<3.0	30	10	32
	14	8.0	<0.3	<3.0	20	20	32
	16	8.0	<0.3	<3.0	20	10	32
	18	8.1	<0.3	<3.0	30	10	32
6 March	12	8.0	<0.3	<3.0	10	10	32
	14	8.0	<0.3	<3.0	10	10	33
	16	8.0	<0.3	<3.0	20	10	34
	18	8.0	<0.3	<3.0	10	10	33

Nitrate levels remained, among the first three sample groups, at or below detectability levels of 10 μg/l. Samples collected during the last two measurement periods (28 February and 6 March) exhibited an increase in nitrate concentration, up to 30 μg/l. Phosphate concentrations varied, across all samples, from a minimum of <10 μg/l (below detectability) to a maximum of 40 μg/l. Phosphate exhibited a general decrease in concentration in the last two sample groups (28 February and 6 March).

Growth Data

Growth measurements were made on three portions of the plants: new blade area, old blade area, and sporophyll area. TABLE 3.8-15 provides a summary of these measurements for all treatments at the beginning of the experiment (day 0). All of the plants retained most of the old blade (previous year's growth) and had initiated new blade growth at the time the experiment was started. Of the 125 plants used in the experiment, only six had begun sporophyll formation.

Histograms showing the data from all observation periods are presented in FIGURE 3.8-10 for old blade surface area. Inspection of this figure reveals that old blade area for the most part did not change with time up to day 21. By days 28 and 35, some decrease in old blade area was evident in all but the 11.9 C treatment. Plants in the 17.9 C treatment appeared to show a consistent gradual decrease in old blade area with each observation period. LSD, Scheffe and SNK procedures were applied to log transformed old blade data, the 11.9 to 17.9 C treatments, from day 35. None of these treatments were significantly different using these tests. A rapid decrease in old blade area was evident in the 19.9 C treatment by day 21, when it was terminated. These data confirm the supposition that the portion of the plants designated as old blade tissue represents blades produced during the previous year and that growth of these blades was permanently terminated prior to the experiment. The decrease in old blade area seen in all but the 11.9 C treatment undoubtedly reflects the natural process of old blade senescence, a well-known phenomenon in this species (Abbott and Hollenberg 1976). These data show that the rate of old blade

TABLE 3.8-15

SUMMARY STATISTICS ON PLANT MEASUREMENTS TAKEN ON DAY 0:
TREE KELP SPOROPHYTE OPTIMUM TEMPERATURE FOR GROWTH

Treatment (C)	Plant Measurements (cm^2)												
	Old Blade (N=25)				New Blade (N=25)				Sporophyll				
	$\bar{X}$	S.D.	Max.	Min.	$\bar{X}$	S.D.	Max.	Min.	$\bar{X}$	S.D.	Max.	Min.	N
11.9	10.8	10.9	42.0	0.8	7.2	5.0	17.2	0.6	–	–	–	–	–
13.9	10.8	9.6	36.9	0.7	6.8	3.4	13.2	1.2	0.2	–	–	–	1
15.8	11.9	7.4	25.9	0.6	5.7	3.0	11.5	0.9	0.4	–	–	–	1
17.9	7.8	6.2	22.8	1.0	9.1	6.2	21.8	1.4	0.5	–	–	–	1
19.9	7.4	4.9	18.1	0.7	7.9	5.0	16.8	1.7	0.1	0.0	0.2	0.1	3

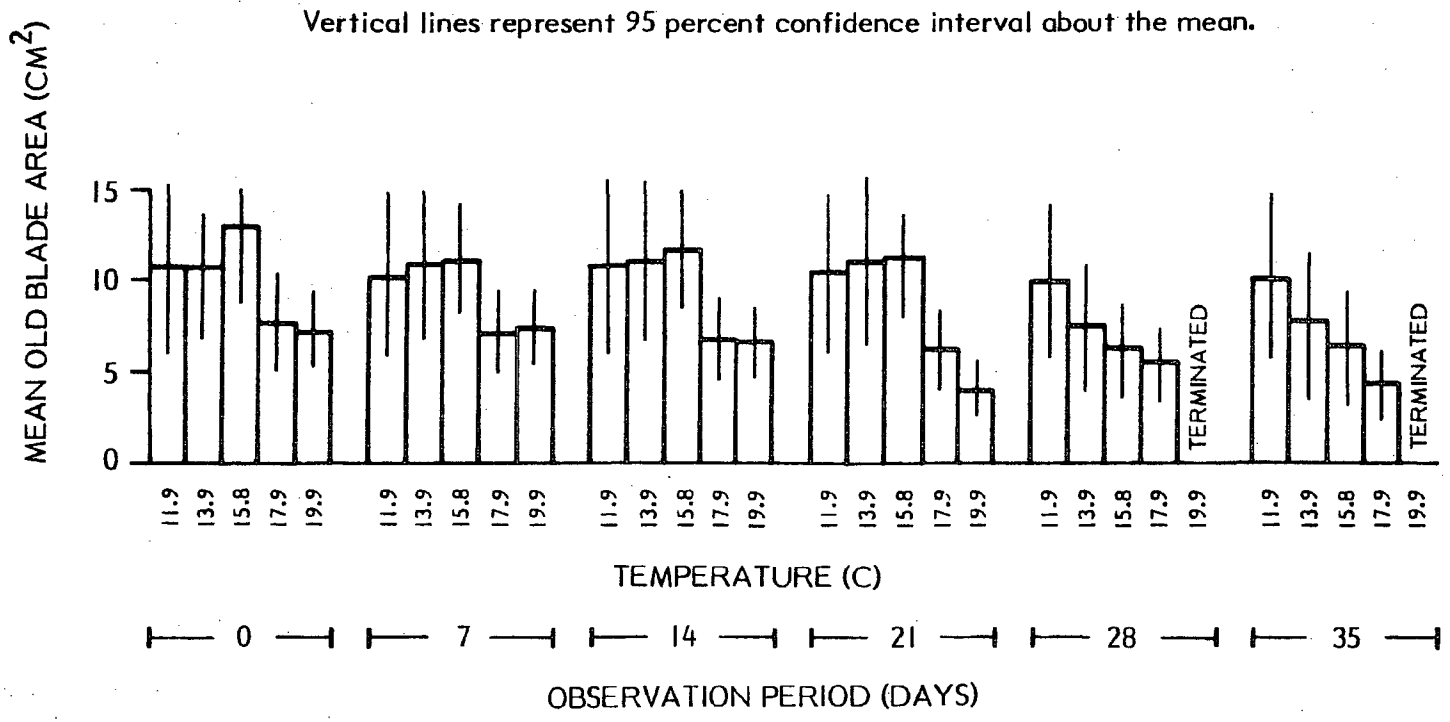


FIGURE 3.8-10

OLD BLADE AREA VERSUS TIME: TREE KELP SPOROPHYTE
OPTIMUM TEMPERATURE FOR GROWTH

senescence increases with increasing temperature, a trend to be anticipated based on general principles of plant physiology.

Most of the growth measured during the experiment occurred in the new blades (FIGURE 3.8-11) with substantial growth occurring in all but the 19.9 C treatment. The optimum temperature for growth of the new blades appears to be well defined at approximately 14 C, with significant (based on inspection of the 95 percent confidence intervals) decreases in growth both above and below this temperature. Examination of the data for the 19.9 C treatment on days 14 and 21 reveals that no growth had occurred in this period. It is likely that 19.9 C represents an upper temperature limit to growth of this species and may possibly represent a lethal temperature in the long term. The trend in the data appeared to follow a logarithmic or exponential function, as expected. The data were transformed to $\log_{10}$ and a multiple regression analysis was performed on these data. The results are shown in FIGURE 3.8-12. These graphs reiterate the conclusions from the histograms: optimum growth rate occurred at 13.9 C and decreased markedly in the 15.8, 11.9, 17.9 and 19.9 C treatments in that order. Three statistical procedures were applied to the new blade data (log transformed) from day 35 (see TABLE 3.8-16). The LSD and Scheffe procedures revealed that, at $p = 0.01$ and 0.05 respectively, the 13.9 C treatment showed significantly greater new blade production than all other treatments, the 15.8 C treatment had a significantly greater new blade production than the 17.9 C treatment and that the 11.9 C treatment did not differ from the 15.8 or 17.9 C treatments. The SNK procedure revealed that the 11.9 and 17.9 C treatments were not significantly different, the 15.8 C treatment showed significantly greater new blade production and the 13.9 C treatment showed significantly more new blade production than all of the other treatments.

Formation of sporophylls occurred on a small portion of the plants during the experiment. As shown in FIGURE 3.8-13, the order of treatments from highest to lowest number of plants with sporophylls corresponds exactly with the order revealed by new blade growth, except for the 19.9 C treatment in which three plants had sporophylls on day 0. Because of the low numbers, it is difficult from these data to state conclusively whether there is a real temperature effect on

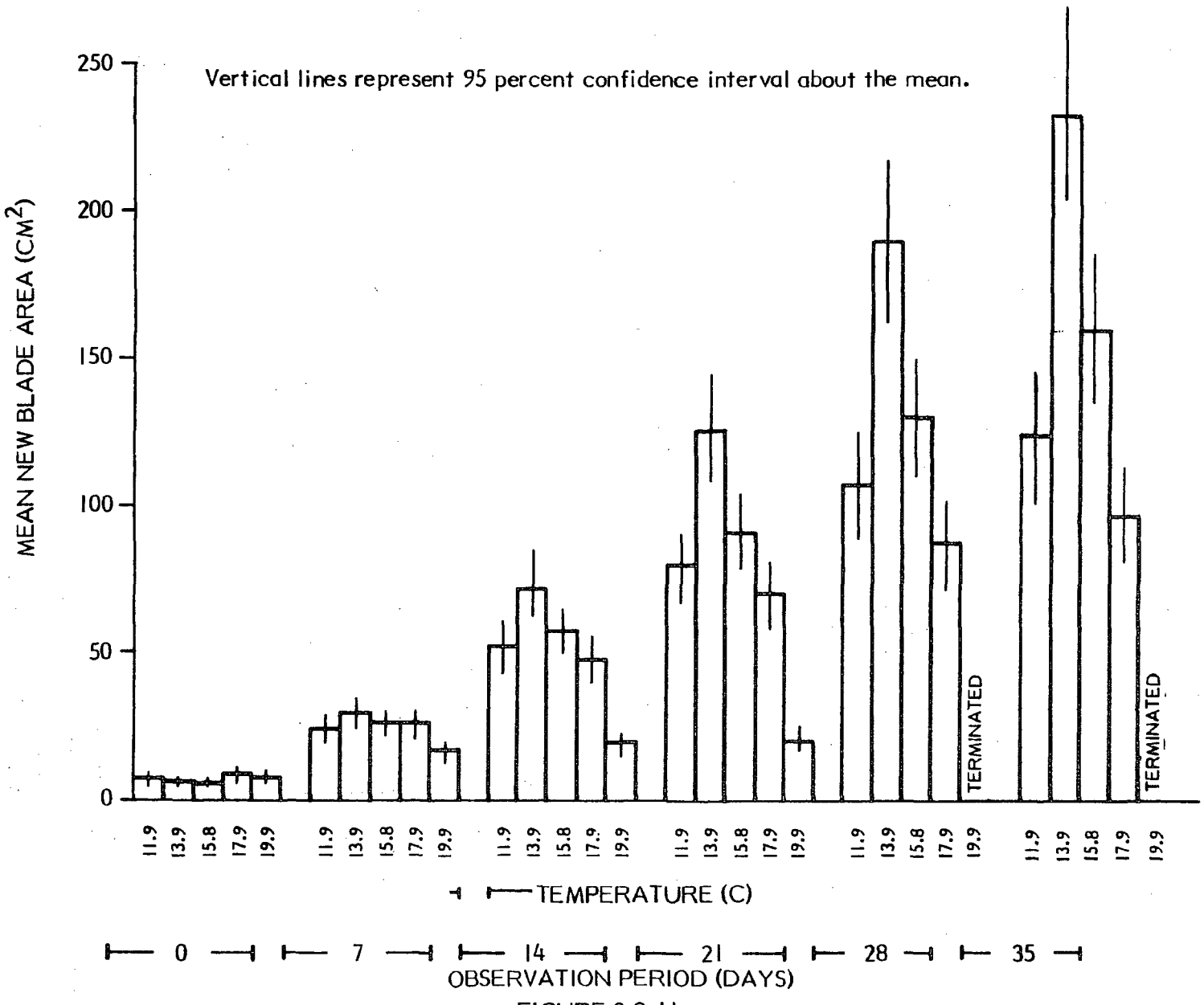


FIGURE 3.8-11

NEW BLADE AREA VERSUS TIME: TREE KELP
SPOROPHYTE OPTIMUM TEMPERATURE FOR GROWTH

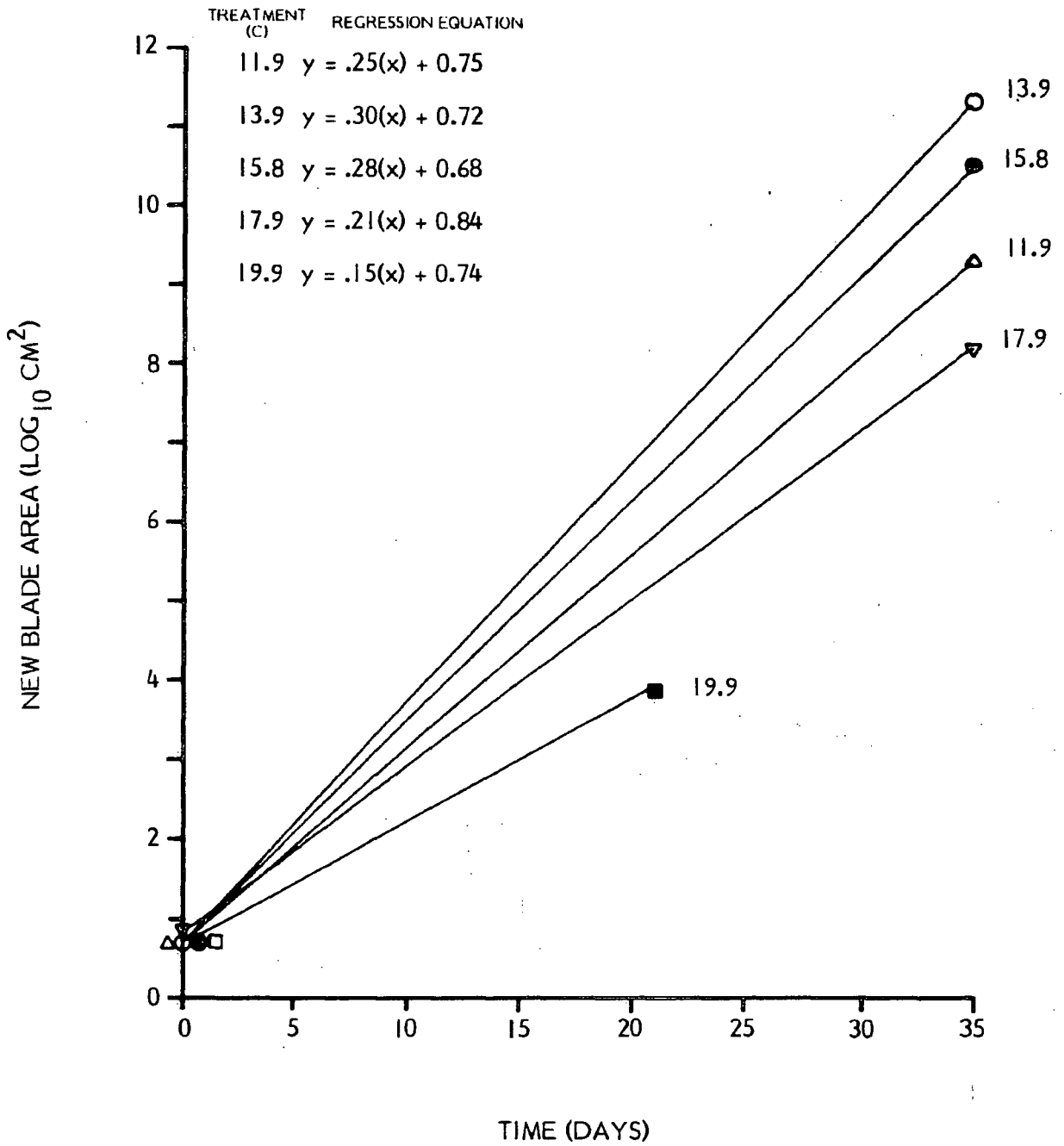


FIGURE 3.8-12

REGRESSION ANALYSIS OF NEW BLADE AREA VERSUS TIME FOR ALL TREATMENTS: TREE KELP SPOROPHYTE OPTIMUM TEMPERATURE FOR GROWTH

TABLE 3.8-16

RESULTS OF STATISTICAL TESTS ON LOG_{10} NEW BLADE AREA (CM^2) AT DAY 35: TREE KELP SPOROPHYTE OPTIMUM TEMPERATURE FOR GROWTH

Treatment (C)	17.9	11.9	15.8	13.9
Mean (Log_{10} cm^2)	1.95	2.04	2.18	2.35

LSD ($p = 0.01$), Scheffe ($p = 0.05$)

Treatment (C)	Significantly Different from
11.9	13.9
13.9	11.9, 15.8, 17.9
15.8	13.9, 17.9
17.9	13.9, 15.8

SNK ($p = 0.05$)		Treatment (C)
subset 1	17.9	11.9
subset 2		15.8
subset 3		13.9

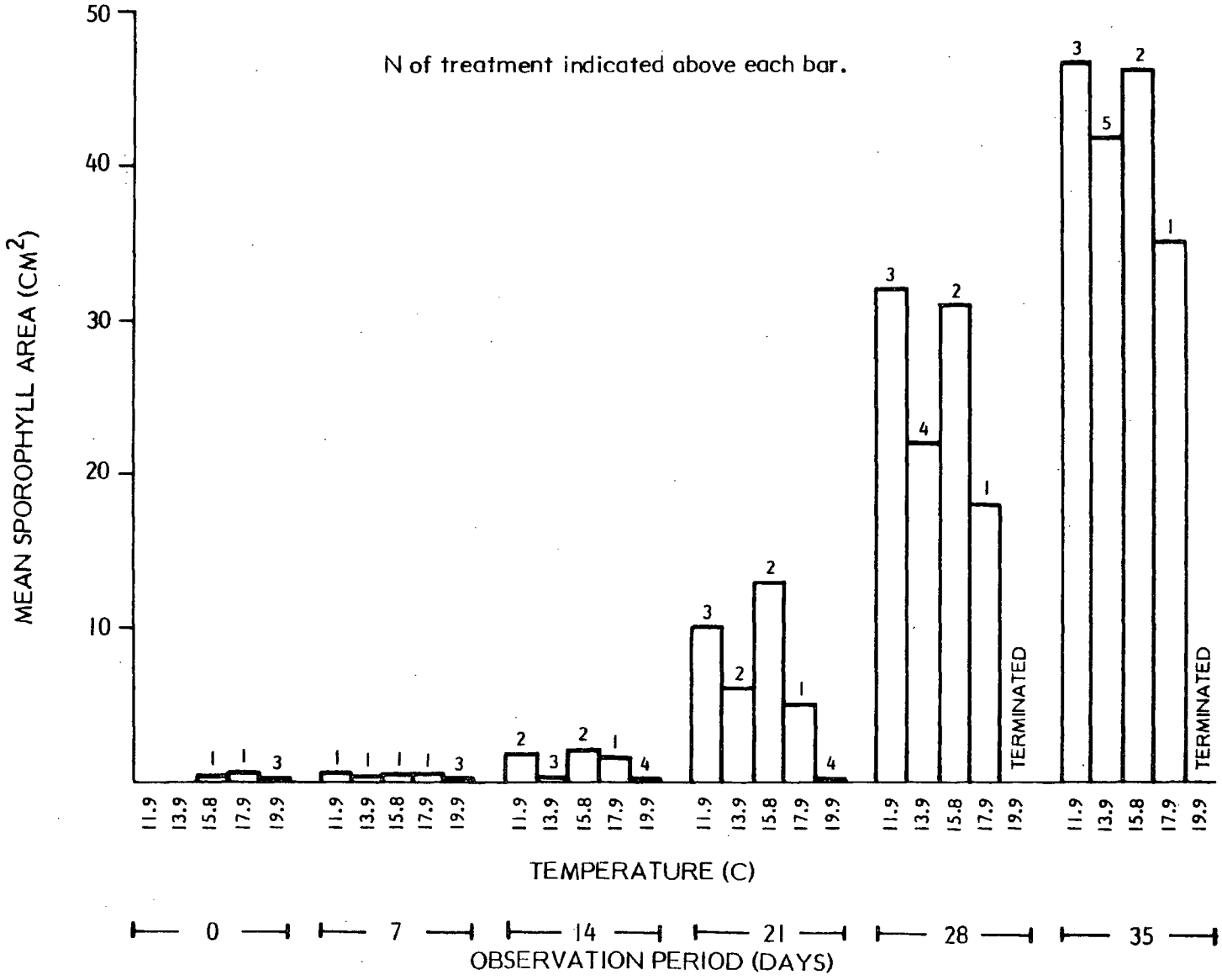


FIGURE 3.8-13

SPOROPHYLL AREA VERSUS TIME: TREE KELP
SPOROPHYTE OPTIMUM TEMPERATURE FOR GROWTH

sporophyll formation and what the effect is. They do show that sporophyll formation can occur at least up to 15.8 C. Sporophyll area was measured and the resulting data are summarized in FIGURE 3.8-13. Growth of sporophylls showed a normal trend (logarithmic or exponential) in all but the 19.9 C treatment. The lack of significant growth at 19.9 C, particularly in view of the relatively high number of sporophylls present on day 0 (see FIGURE 3.8-13), indicate that this temperature completely inhibits sporophyll growth.

3.8.3.3 DISCUSSION

The optimum temperature for growth of tree kelp sporophytes was determined to be approximately 14 C (57 F). Rate of growth decreases markedly as temperature decreases and increases from this value. At and above 19.9 C (67.8 F), growth of new blade tissue and sporophylls (the reproductive portion of the plant) is inhibited.

3.9 IRIDESCENT SEAWEED (IRIDAEA FLACCIDA)

A detailed review of the taxonomy, ecology, life history and thermal biology of the iridescent seaweed (Iridaea flaccida, was presented by PGandE (1979b). To orient the reader, a brief summary of the pertinent aspects of its biology are presented here.

In the Diablo Canyon area iridescent seaweed is "the most abundant and ubiquitous intertidal alga," especially in the mid and low intertidal zones (Burge and Schultz 1973). Abundance of iridescent seaweed declines in the winter (Burge and Schultz 1973); however, fertile material is present then as it is throughout the rest of the year (K. Carr, 316(a), personal observation).

Reproduction in iridescent seaweed involves three types of plants and two kinds of spores. One type of plant, the gametophyte, occurs in the field as conspicuous male or female plants. Fertilization of the female plant by non-motile gametes from the male results in the second type of plant, the carposporophyte. This plant, which develops in and is retained on the female and is obvious only as bumps on the blade surface, produces carpospores, which are released from the female blade. Germination and development of carpospores results in the third type of plant, the tetrasporophyte which is also conspicuous in nature. These plants produce tetraspores, which are released from the blade and develop into male or female plants, completing the cycle.

Results from four experiments are presented in this section. The first two experiments examined the effects of temperature on early development of gametophytes and sporophytes. The second two experiments were concerned with temperature effects on growth of cultured and field-collected plants.

3.9.1 GAMETOPHYTE GERMINATION AND EARLY DEVELOPMENT

The results of an experiment to determine the minimum, optimum and maximum temperatures for early development of the red alga Iridaea flaccida are presented in this section. The experiment was conducted on the gametophytic

generation. The results of an identical experiment conducted on the tetrasporophytic generation of I. flaccida are reported in the next section, and the results from an experiment conducted on the gametophytic generation of the closely related I. cordata are reported in SECTION 3.10.6.

The experiment described in this section follows the germination and early development of tetraspores into male and female gametophytes. Typically, development occurs by division of the spherical spore into several cells, usually with no increase in spore size. Subsequent cell divisions form a disc of cells, which increases in diameter as growth continues. Expansion of the disc eventually diminishes as upright blades begin to grow from it. Measurements of discs before blade formation can be used to assess initial growth.

The study objective was to determine the minimum, optimum and maximum temperatures for early development and initial growth of iridescent seaweed gametophytes. The purpose was to provide an estimation of the effects of the power plant thermal discharge on recruitment and initial development of iridescent seaweed in Diablo Cove.

3.9.1.1 METHODS

A summary description of laboratory methods employed in the investigations into minimum, optimum and maximum temperatures for early development of iridescent seaweed gametophytes is presented below. For a more detailed description of the laboratory methods see APPENDIX B, Procedure 321, and protocol (see APPENDIX C, experiment 21a).

Tetrasporangial plants were collected from an intertidal area south of the power plant intake on 20 August 1979. They were transported to the laboratory in plastic bags and held in ambient seawater prior to use for the experiment. Before spore release, the plants were briefly scrubbed in 2 percent bleach in seawater and rinsed with sterile seawater. Spore release was induced by drying pieces of the plants at room temperature (20 C) for 5 minutes and then immersing them in sterile seawater. A stirred suspension of spores was used to

establish cultures on coverslips, which were then incubated about 6 hours to allow the spores to attach.

Cultures were placed on the gradient tables at the temperatures and light intensities specified in the experiment protocol (see APPENDIX C, experiment 21a). One coverslip was removed from each culture after 1, 3, 6, 9 and 15 days on the gradient table and random number pairs were used to locate 30 sporelings for measurement.

Size of the sporeling discs was measured as diameter (in μm) and converted to area (μm^2), assuming that disc shape was a perfect circle as observed from above. Visual observations confirmed that sporeling discs nearly always approximated a circle in shape when normally developed. Cell number was also recorded until growth perpendicular to the substrate surface precluded an accurate count. This usually occurred after day 3 so that these counts are available only for the first two observation periods (days 1 and 3).

3.9.1.2 RESULTS

A complete summary of the data and analytical results is presented in this section. Copies of all original raw data sheets are filed on the Diablo Canyon site and other locations as hard copy and microfiche.

Temperature Data

Temperature data for the entire experiment are summarized in TABLE 3.9-1. The mean of the means calculated for the three light intensities at each target temperature is given in the column headed "mean of means" and this value is referred to in the following text, tables and figures for simplicity. Although the means for target temperatures of 18 and 19 C were very close (17.4 and 17.5 C, respectively), the range of temperatures recorded for each of these target temperatures differs. The low mean for the 19 C target appears to have been caused by a change in the heating and cooling characteristics of the gradient. After nine days into the experiment, the maximum temperatures recorded for

TABLE 3.9-1

SUMMARY OF TEMPERATURE DATA:
IRIDESCENT SEAWEED OPTIMUM TEMPERATURE
FOR EARLY DEVELOPMENT OF GAMETOPHYTES

Target Temperature (C)	Target Light Intensity ($\mu E/m^2/s$)	Actual Temperature			
		Mean (S.D.)	Maximum	Minimum	Mean of means
9	110	9.4 (0.17)	9.7	9.0	9.4
	165	9.4 (0.19)	9.7	8.9	
	220	9.5 (0.23)	9.9	9.1	
13	110	12.7 (0.60)	13.6	11.4	12.5
	165	12.5 (0.61)	13.4	11.2	
	220	12.6 (0.65)	13.5	11.2	
16	110	15.0 (0.92)	16.5	13.0	15.1
	165	15.0 (0.94)	16.5	12.9	
	220	15.2 (0.95)	16.7	13.1	
18	110	17.4 (0.84)	18.4	16.0	17.4
	165	17.4 (0.80)	18.3	16.0	
	220	17.4 (0.83)	18.3	15.9	
19	110	17.4 (1.16)	19.3	14.5	17.5
	165	17.6 (1.24)	19.7	14.6	
	220	17.5 (1.23)	19.4	14.5	
20	110	19.4 (0.96)	20.5	17.7	19.4
	165	19.4 (0.94)	20.5	17.7	
	220	19.4 (0.98)	20.5	17.7	
22	110	22.2 (0.66)	23.0	20.7	22.3
	165	22.5 (0.74)	23.4	20.9	
	220	22.3 (0.72)	23.1	20.6	

the 19 C target dishes dropped from the 19.3 to 19.7 C range to a range of 18.2 to 18.5 C and remained within the latter range until the end of the experiment.

The mean number of cells per sporeling for each treatment at days 1 and 3 is given in TABLE 3.9-2 and is shown graphically in FIGURE 3.9-1. At day 1, cell number was rather uniform between 12.3 and 19.4 C. Cell number at both 9.4 and 22.3 C was slightly lower. Between days 1 and 3, cell number increased noticeably at temperatures between 15.1 and 19.4 C, with peak values at 17.5 C for all light intensities. Cell number increased at a slower rate at 9.4 and 12.5 C, while the smallest increase from day 1 to 3 occurred at 22.3 C. Light intensity appeared to have very little effect on cell number.

Sporeling Growth Through Time

The log of the sporeling area was plotted against time and a regression line was calculated for each treatment (see FIGURES 3.9-2, 3.9-3, and 3.9-4 and TABLE 3.9-3). The slopes of the regression lines indicate relative differences in growth rate between conditions. For all three light intensities the lowest rate of growth occurred at 22.3 C. Whether or not the spores actually grew at 22.3 C is questionable. Some of the spores were still pigmented by the end of the experiment, but in most there were only 3-4 cells per sporeling and the shapes of the cells were abnormal (FIGURE 3.9-5). Some sporelings at 19.4 C were irregular in outline but the "normal" circular sporelings were also present. Sporelings at the other temperatures were more or less circular in outline with a peripheral zone of colorless cells (where active cell division occurs) and a center of darker red cells.

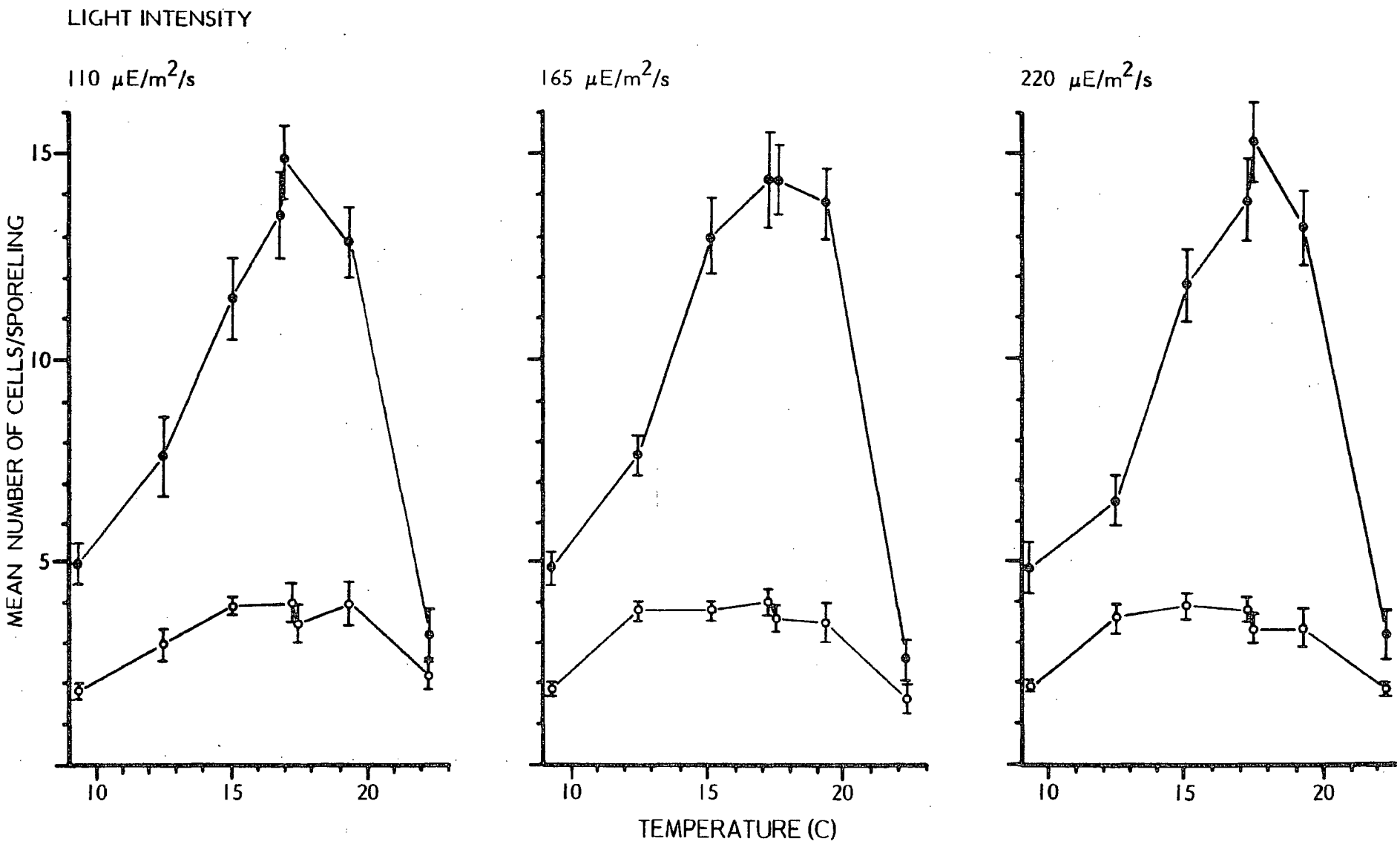
A two-way analysis of variance was performed on the log area at day 15 for all the experimental conditions, except those at 22.3 C. The results of this analysis are shown in TABLE 3.9-4. The temperature effect is highly significant while that of light intensity is much lower. The interaction of these two factors is not significant. Individual means were compared using the Least Significant Difference (LSD) method and the results of this test are shown in FIGURE 3.9-6.

TABLE 3.9-2

MEAN NUMBER OF CELLS PER SPORELING:
IRIDESCENT SEAWEED OPTIMUM TEMPERATURE FOR
EARLY DEVELOPMENT OF GAMETOPHYTES

(Standard Deviations in Parentheses)

Mean Temperature (C)	Target Light Intensity ($\mu E/m^2/s$)	Days 1		Days 3	
9.4	110	1.8	(0.4)	4.9	(1.6)
	165	1.8	(0.4)	4.8	(1.1)
	220	1.9	(0.3)	4.8	(1.6)
12.5	110	2.9	(1.0)	7.7	(2.7)
	165	3.8	(0.6)	7.7	(1.5)
	220	3.6	(0.8)	6.5	(1.6)
15.1	110	3.9	(0.3)	11.5	(2.8)
	165	3.8	(0.6)	13.0	(2.5)
	220	3.9	(0.7)	11.8	(2.5)
17.4	110	4.0	(1.3)	13.5	(2.9)
	165	4.0	(0.8)	14.4	(3.2)
	220	3.8	(0.8)	13.9	(2.8)
17.5	110	3.5	(1.0)	14.8	(2.6)
	165	3.6	(0.8)	14.4	(2.6)
	220	3.4	(1.2)	15.3	(2.9)
19.4	110	4.0	(1.1)	12.9	(2.6)
	165	3.5	(1.3)	13.8	(2.6)
	220	3.4	(1.3)	13.2	(2.5)
22.3	110	2.2	(0.8)	3.2	(1.6)
	165	1.6	(0.7)	2.6	(1.3)
	220	1.8	(0.6)	3.2	(1.8)



Vertical bars are 95 percent confidence limits (N = 30).

FIGURE 3.9-1

MEAN NUMBER OF CELLS PER SPORELING AT DAY 1 (-o-): IRIDESCENT SEAWEED OPTIMUM TEMPERATURE FOR EARLY DEVELOPMENT OF GAMETOPHYTES

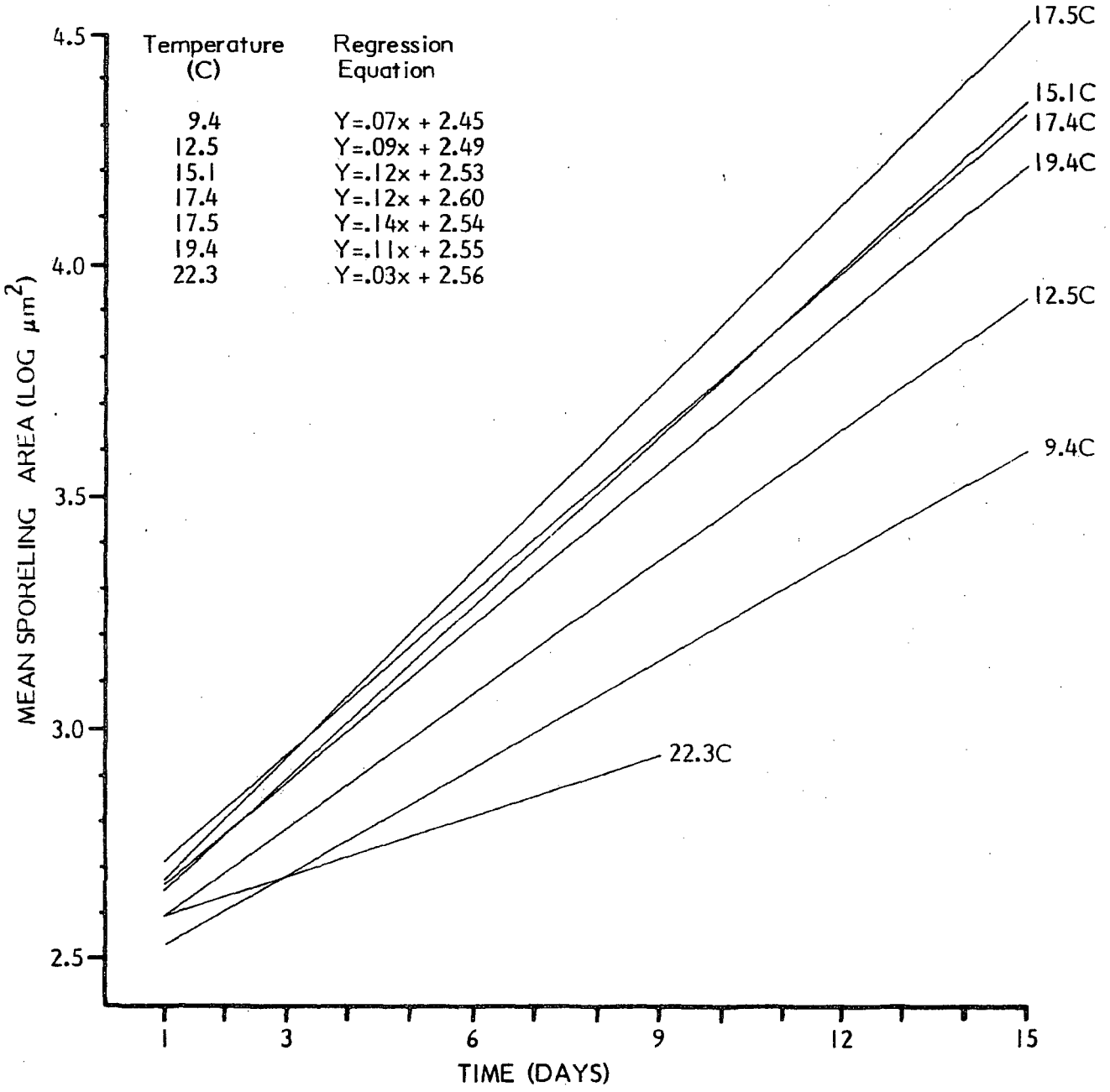


FIGURE 3.9-2

SPORELING DISC AREA VERSUS TIME AT 100 $\mu E/m^2/s$: IRIDESCENT SEAWEED OPTIMUM TEMPERATURE FOR EARLY DEVELOPMENT OF GAMETOPHYTES

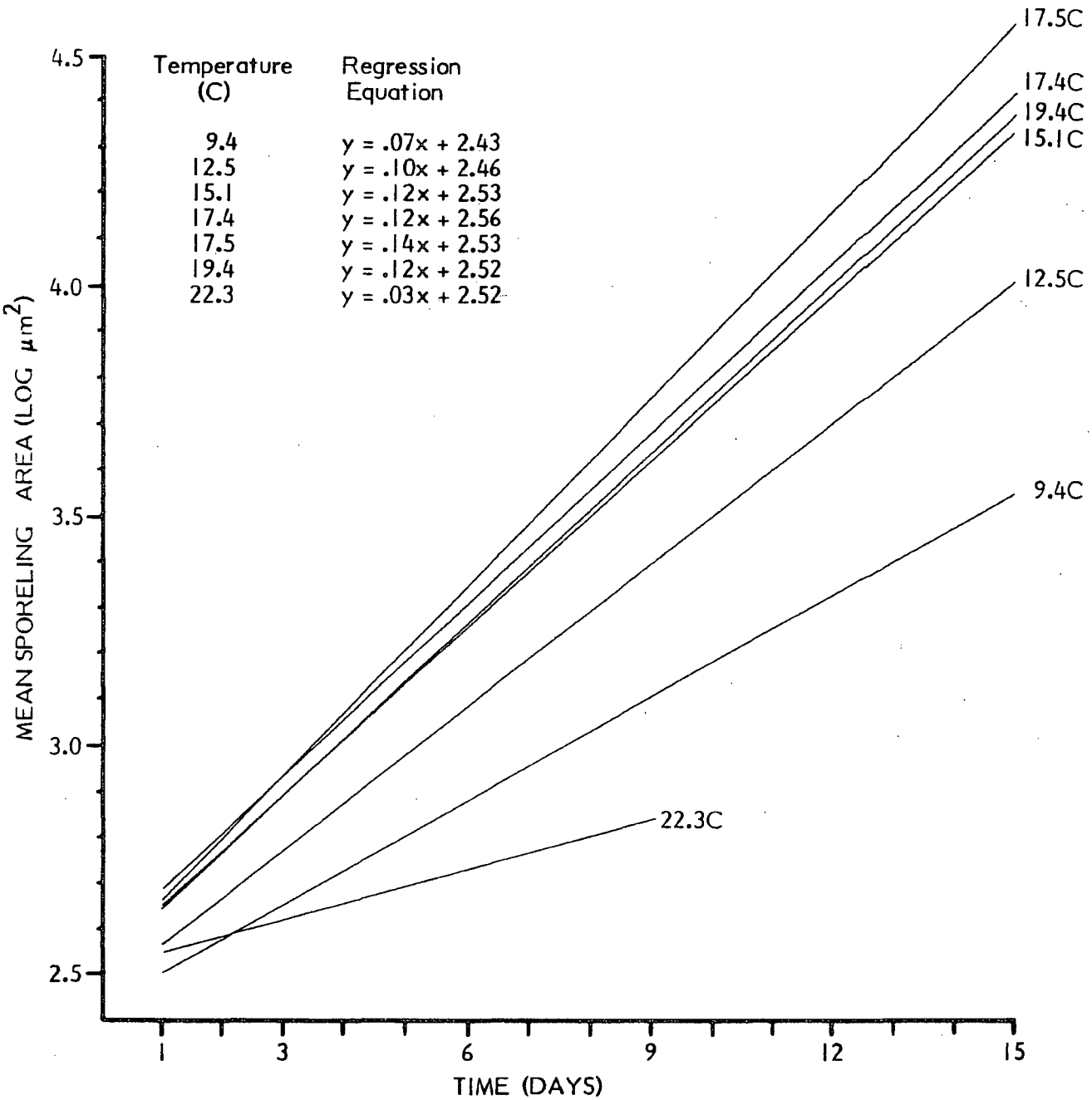


FIGURE 3.9-3

SPORELING DISC AREA VERSUS TIME AT 165 μ E/m^2/s: IRIDESCENT SEAWEED OPTIMUM TEMPERATURE FOR EARLY DEVELOPMENT OF GAMETOPHYTES

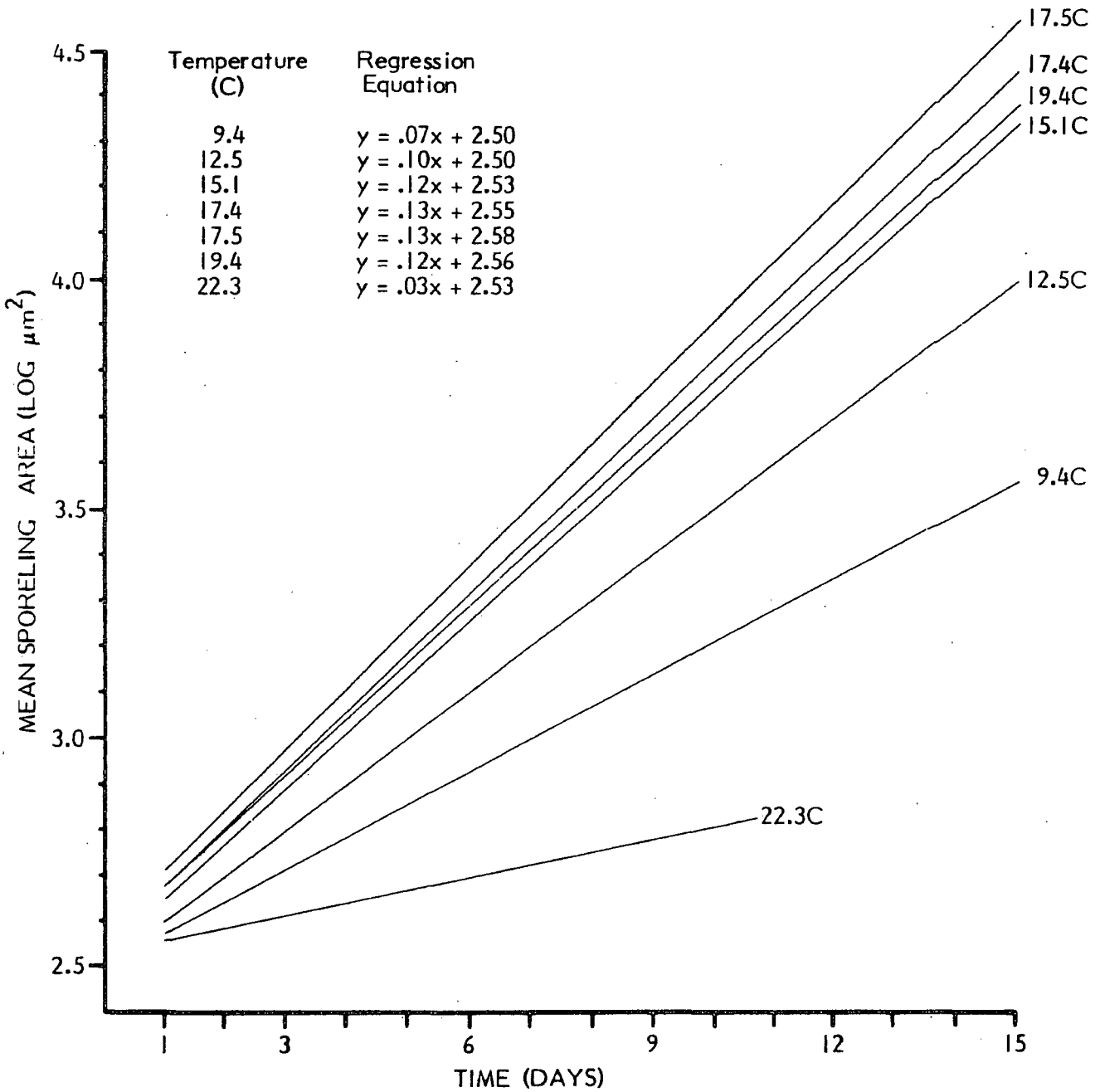


FIGURE 3.9-4

SPORELING DISC AREA VERSUS TIME AT 220 $\mu E/m^2/s$: IRIDESCENT SEAWEED OPTIMUM TEMPERATURE FOR EARLY DEVELOPMENT OF GAMETOPHYTES

TABLE 3.9-3

LOG$_{10}$ OF MEAN SPORELING DISC AREA (μm^2)
AT DAYS 1, 3, 6, 9 AND 15:
IRIDESCENT SEAWEED OPTIMUM TEMPERATURE FOR
EARLY DEVELOPMENT OF GAMETOPHYTES

Cultures in which erect blade initiation was observed are indicated by "*".

Temperature (C)	Days 1	3	6	9	15
Light Intensity: 110 $\mu E/m^2/s$					
9.4	--	2.63	2.92	3.15	3.58
12.5	2.63	2.67	3.08	3.39	3.96
15.1	2.56	2.80	3.24	3.71	4.27*
17.4	2.65	2.83	3.34	3.72	4.31*
17.5	2.58	2.88	3.37	3.84	4.50*
19.4	2.54	2.88	3.28	3.56	4.21
22.3	2.58	2.67	2.79	2.88	--
Light Intensity: 165 $\mu E/m^2/s$					
9.4	--	2.61	2.91	3.14	3.58
12.5	2.63	2.66	3.13	3.46	4.02
15.1	2.58	2.80	3.31	3.70	4.35*
17.4	2.64	2.80	3.36	3.87	4.51*
19.4	2.58	2.86	3.27	3.72	4.34
22.3	2.54	2.67	2.71	2.84	--
Light Intensity: 220 $\mu E/m^2/s$					
9.4	2.67	2.65	2.92	3.14	3.60
12.5	2.63	2.63	3.12	3.48	3.99
15.1	2.61	2.77	3.29	3.70	4.33*
17.4	2.58	2.89	3.34	3.79	4.45*
17.5	2.58	2.93	3.41	3.90	4.53*
19.4	2.59	2.87	3.31	3.72	4.33
22.3	2.53	2.67	2.76	2.77	--

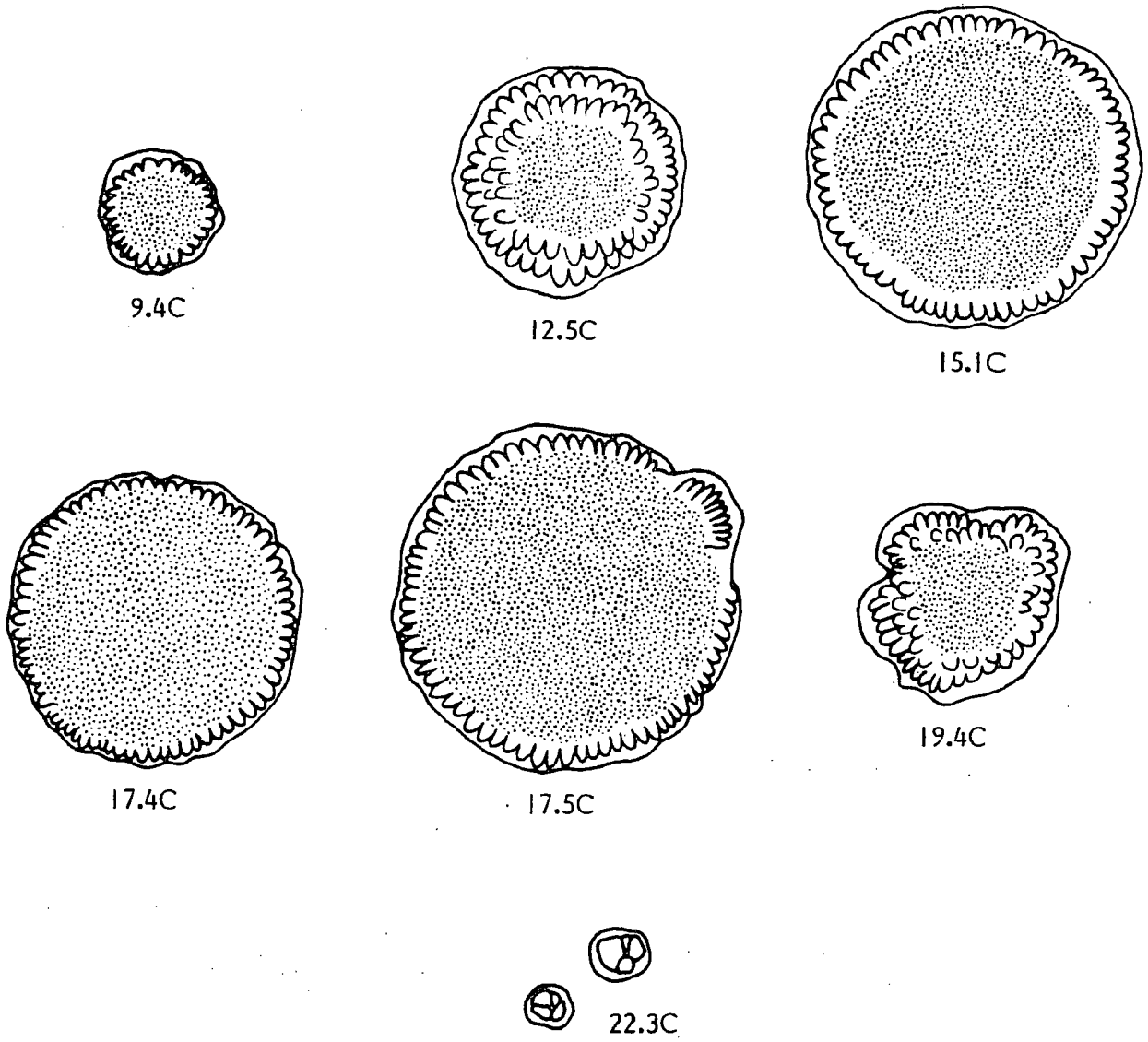


FIGURE 3.9-5

SPORELING DISC SIZE AT DAY 15:
IRIDESCENT SEAWEED FOR EARLY
DEVELOPMENT OF GAMETOPHYTES

Examples taken from 110 $\mu E/m^2/s$ light level. Cells of peripheral colorless zone drawn. Central dark red areas shaded.

TABLE 3.9-4

TWO-WAY ANALYSIS OF VARIANCE ON LOG_{10} SPORELING DISC AREA AT DAY 15: IRIDESCENT SEAWEED OPTIMUM TEMPERATURE FOR EARLY DEVELOPMENT OF GAMETOPHYTES

Sums of X

Light Intensity $\mu E/m^2/s$	Temperature (C) 9.4	12.5	15.1	17.4	17.5	19.4	$\sum$
110	106.96	117.41	126.81	128.37	134.81	126.10	740.46
165	107.07	118.90	129.44	131.60	135.36	130.05	752.42
220	107.77	118.55	128.89	132.03	135.75	129.93	752.92
$\sum$	321.80	354.86	385.14	392.00	405.92	386.08	2,245.8

$\sum x^2 = 9{,}417.32$

ANOVA

Source	df	SS	MS	F_{CALC}	F_{TAB}
Subgroups	17	53.06			
Temperature	5	52.26	10.45	225.22	3.02
Light Intensity	2	0.55	0.276	5.96	4.61
Interaction	10	0.25	0.025	0.53	2.32
Error	522	24.23	0.046		
Total	539	77.29			

$$LSD = 2.576\sqrt{2/30 \bullet 04} = 0.143$$

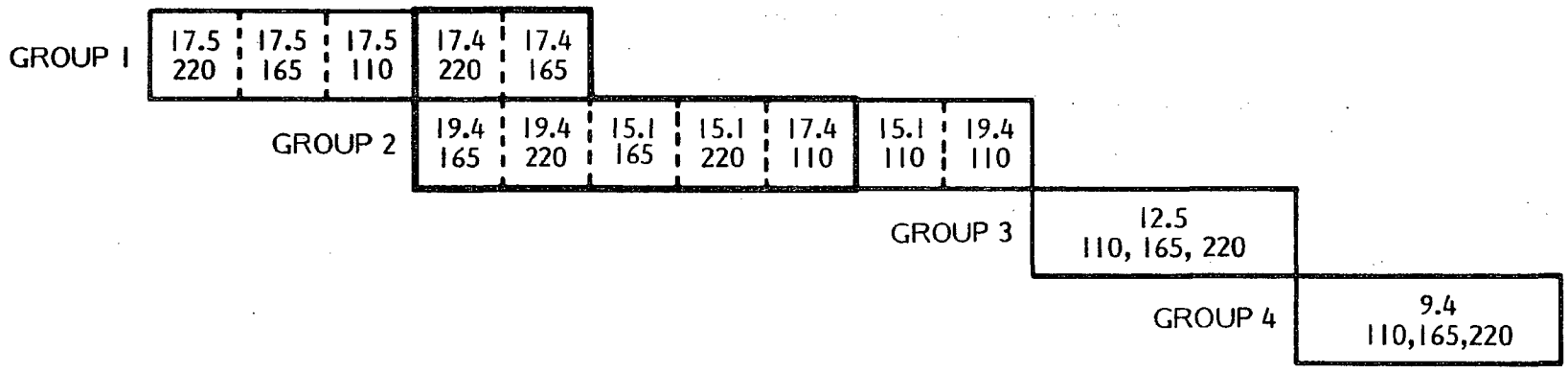


FIGURE 3.9-6

RESULTS OF LSD TEST ON LOG_{10} SPORELING DISC AREA AT DAY 15: IRIDESCENT SEAWEED OPTIMUM TEMPERATURE FOR EARLY DEVELOPMENT OF GAMETOPHYTES

Numbers in squares are temperatures (C) (top) and light intensities ($\mu E/m^2/s$ bottom). Log areas decrease from left to right and from top to bottom. Members of groups are statistically the same ($p = .01$). The heavy lines around squares in Groups 1 and 2 indicate overlap. Groups 3 and 4 are distinct.

From this test it is evident that the only significant light intensity effects occurred at 15.1 and 19.4 C, where the 110 $\mu E/m^2/s$ plants were smaller than those at higher light intensities. Light intensity effects are not statistically detectable at the other temperatures. Peak development occurs at 17.5 C for all three light levels, with some overlap with 17.4 C. However, the decrease in growth from 17.5 to 19.4 C is significant for all light levels. Plants at both 12.5 and 9.4 C are significantly smaller than plants at higher temperatures.

Erect Blade Initiation

Initiation of erect blades from the sporeling crusts was observed in some of the treatments only on day 15 (TABLE 3.9-3). Only in the 15.1, 17.4 and 17.5 C treatments (at all three light intensities) were erect blades initiated. A plot of blade initiation versus growth and temperature is shown in FIGURE 3.9-7. Generally, blade initiation appeared to occur when disc area reached about 15,850 μm^2, except in the 19.4 C treatments.

3.9.1.3 DISCUSSION

The optimum temperature for early development of iridescent seaweed gametophytes in culture is about 17.4 C (63.3 F), based upon increases in sporeling cell number and disc area over 15 days. Marked decreases in rate of development were observed as temperature decreased from 15.1 to 12.5 C (59.2 to 54.5 F) and as temperature increased from 19.4 to 22.3 C (66.9 to 72.1 F). Very low rates of development and abnormal morphology characterized the sporelings or 22.3 C (72.1 F), which indicates that this temperature is near or at the upper limit for normal development of iridescent seaweed gametophyte sporelings. Excluding the 22.3 C (72.1 F) treatments, the size of sporelings at 15 days was lowest at 9.4 C (48.9 F) and next lowest at 12.5 C (54.5 F). The next larger sporeling sizes generally included the 15.1 and 19.4 C (59.2 and 66.9 F) treatments but there was considerable overlap with the 17.4 and 17.5 C (63.3 and 63.5 F) treatments in which the largest sporelings occurred. Light intensity appeared to have no significant differential effect on growth in this experiment.

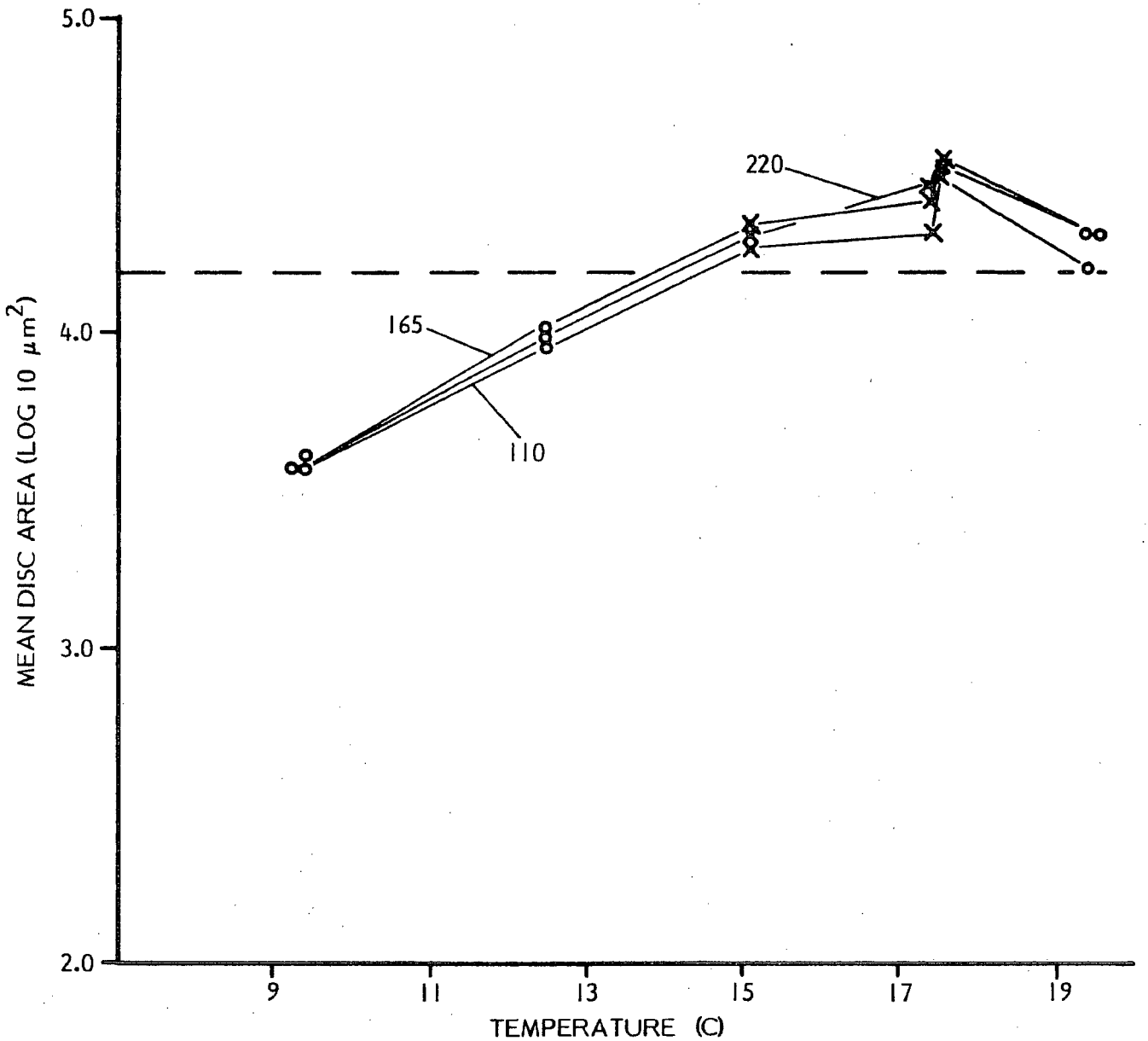


FIGURE 3.9-7

BLADE INITIATION RELATIVE TO SPORELING DISC AREA, TEMPERATURE AND LIGHT INTENSITY AT DAY 15: IRIDESCENT SEAWEED OPTIMUM TEMPERATURE FOR EARLY DEVELOPMENT OF GAMETOPHYTES

Light intensities shown as $\mu E/m^2/s$. "X" = blade initiation observed "0" = blade initiation not observed. Horizontal dashed line at 4.2 (log 10) μm^2 level.

Initiation of erect blades was observed at the last observation (15 days) only in the 17.4, 17.5 and 15.1 C (63.3, 63.5 and 59.2 F) treatments. It is interesting to note that the sizes of sporelings in the 15.1 C (59.2 F) treatments were not statistically different from those at 19.4 C (66.9 F). The lack of blade initiation in some of the treaments at 15 days most likely indicates a delay of initiation compared to the 17.4, 17.5 and 15.1 C (63.3, 63.5 and 59.2 F) treatments rather than complete suppression.

3.9.2 SPOROPHYTE EARLY DEVELOPMENT

The results of an experiment to determine the minimum, optimum and maximum temperatures for early development of the iridescent seaweed are presented in this section. The experiment was conducted on the sporophytic generation. The results of an identical experiment conducted on the gametophytic generation of iridescent seaweed were reported in the previous section and the results from an experiment conducted on the gametophytic generation of the closely related I. cordata are reported in SECTION 3.10.6. The experiment described in this section involves the early development of iridescent seaweed sporophytes from carpospores. The basic difference between the sporophytic and gametophytic generations is in the chromosome number; cells of gametophytes contain half the number of chromosomes as those of sporophytes. Otherwise, aside from slight differences in size and morphology, the two generations are vegetatively identical.

The study objective was to determine the minimum, optimum and maximum temperatures for early development and initial growth of iridescent seaweed sporophytes. The purpose was to provide an estimation of the effects of the power plant thermal discharge on recruitment and initial development of iridescent seaweed in Diablo Cove.

3.9.2.1 METHODS

A summary description of laboratory methods employed in the investigations into minimum, optimum and maximum temperatures for early development of irides-

cent sporophytes is presented below. For a more detailed description of the laboratory methods see APPENDIX B, Procedure 321, and protocol (see APPENDIX C, experiment 21b).

Fertile plants were collected from an area south of the power plant intake on 16 October 1979 and transported to the laboratory in plastic bags. They were held in ambient seawater prior to use in the experiment. Treatment of the plants prior to spore release was as described in SECTION 3.9.1.1 except that the plants were dried overnight in covered petri dishes at 13 C. The following morning any spores that had discharged during the night were rinsed off and the plants were immersed in sterile seawater. Cultures were established as described in SECTION 3.9.1.1 and were placed on the gradient tables at the temperatures and light intensities specified in the experiment protocol (see APPENDIX C, experiment 21b). Cultures were sampled after 1, 3, 6, 9, 12 and 15 days on the gradient tables and 30 sporelings per treatment were measured at each sample time as described in SECTION 3.9.1.1 except that the number of cells per sporeling was taken on day 1 only.

Size of the sporeling discs was measured as diameter (in μm) and converted to area (μm^2), assuming that disc shape was a perfect circle as observed from above. Visual observations confirmed that sporeling discs nearly always approximated a circle in shape when normally developed. Cell number was also recorded until growth perpendicular to the substrate surface precluded an accurate count.

3.9.2.2 RESULTS

A complete summary of the data and analytical results is presented in this section. Copies of all original raw data sheets are filed on the Diablo Canyon site and other locations as hard copy and microfiche.

Temperature Data

A summary of the temperature data is presented in TABLE 3.9-5. The mean of the means calculated for the three light intensities at each target temperature is given as "mean of means" and this value is referred to in the following presentation of results.

Number Of Cells Per Sporeling

Counts of total number of cells per sporeling were made on day 1 only. By day 3, cell divisions perpendicular to the substrate produced surface cells that obscured the underlying cells, making accurate counts impossible. Analysis of the day 1 data did not appear warranted because they represent only one observation after a very short period of exposure to test conditions.

Sporeling Growth Through Times

Measurements of the diameter of the sporelings were used to calculate the area, which was converted to a log value and plotted against time. Linear regression was used to fit a line for each light intensity and temperature combination (FIGURES 3.9-8, 3.9-9 and 3.9-10 and TABLE 3.9-6). The slopes of these lines indicate relative differences in growth rate between the treatments. The lowest growth rate was observed at 22.3 C for all light intensities. Sporeling observations were discontinued at day 12 for the 22.3 C treatments. At that time, many sporelings had lost all pigmentation although there were still a few pigmented sporelings. The size and shape of the cells were also abnormal (FIGURE 3.9-11).

A one-way analysis of variance of log area versus temperature was performed for each light intensity (excluding the 22.3 C treatments). The results of this test, presented in TABLE 3.9-7, show a significant temperature effect at all light intensities. The Student-Newman-Keuls multiple range test (SNK) was used to compare mean values. The results of this test are presented in TABLE 3.9-8. Plants at 9.0 and 12.3 C were significantly smaller than plants at the higher temperatures. As light intensity increased, however, there was a shift in the ranking of treatments in the 15.1 to 19.3 C range.

TABLE 3.9-5

SUMMARY OF TEMPERATURE DATA:
IRIDESCENT SEAWEED OPTIMUM TEMPERATURE
FOR EARLY DEVELOPMENT OF SPOROPHYTES

Target Temperature (C)	Target Light Intensity ($\mu E/m^2/s$)	Actual Temperature			
		Mean (S.D.)	Maximum	Minimum	Mean of means
9	110	8.9 (0.22)	10.1	8.6	9.0
	165	9.0 (0.26)	10.1	8.6	
	220	9.0 (0.28)	10.2	8.5	
13	110	12.3 (0.60)	13.6	10.9	12.3
	165	12.3 (0.62)	13.5	10.8	
	220	12.4 (0.68)	13.5	11.3	
16	110	15.0 (0.87)	16.2	12.5	15.1
	165	15.0 (0.87)	16.2	12.9	
	220	15.2 (0.88)	16.5	12.8	
18	110	17.4 (0.96)	18.3	15.7	17.3
	165	17.2 (0.90)	18.3	15.5	
	220	17.3 (0.97)	18.3	15.7	
19	110	18.0 (1.16)	19.4	14.1	18.1
	165	18.2 (1.16)	19.6	14.1	
	220	18.2 (1.18)	19.6	14.0	
20	110	19.3 (1.14)	20.6	17.2	19.3
	165	19.3 (1.10)	20.6	17.3	
	220	19.2 (1.08)	20.5	17.4	
22	110	22.2 (0.82)	23.1	20.4	22.3
	165	22.5 (0.88)	23.7	20.5	
	220	22.3 (0.87)	23.6	20.4	

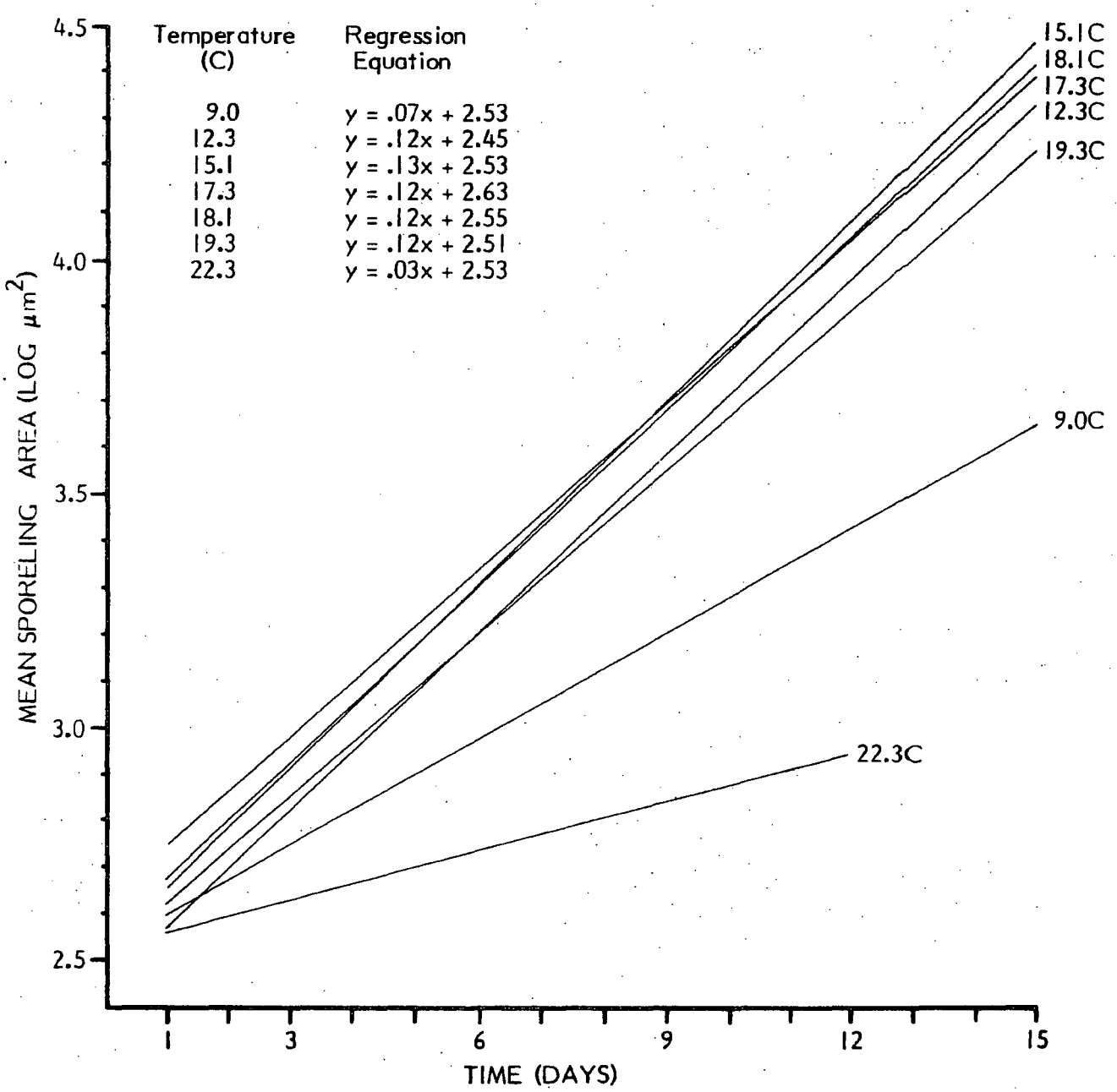


FIGURE 3.9-8

SPORELING DISC AREA VERSUS TIME AT 110 $\mu E/m^2/s$: IRIDESCENT SEAWEED OPTIMUM TEMPERATURE FOR EARLY DEVELOPMENT OF SPOROPHYTES

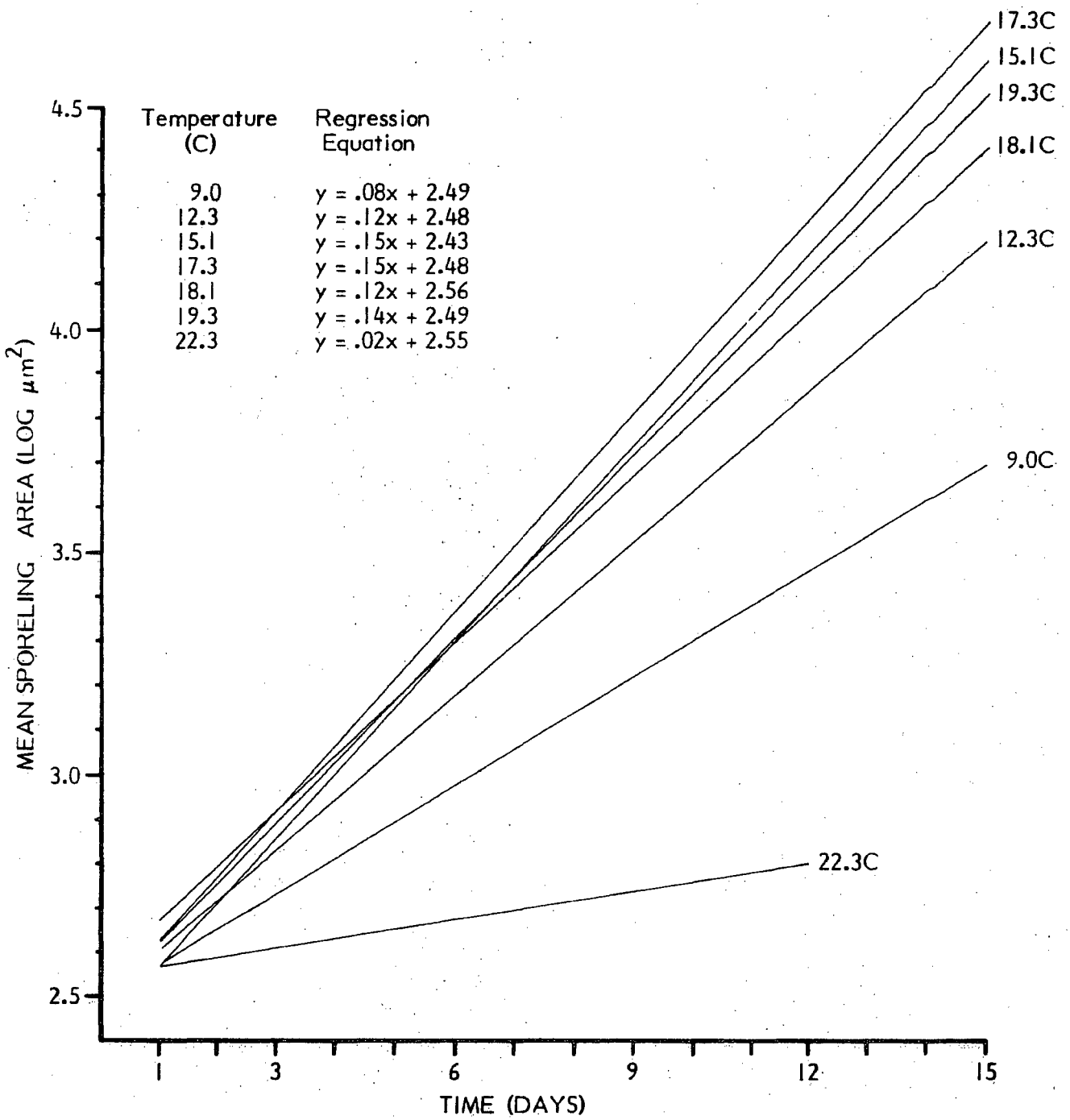


FIGURE 3.9-9

SPORELING DISC AREA VERSUS TIME AT 165 $\mu E/m^2/s$: IRIDESCENT SEAWEED OPTIMUM TEMPERATURE FOR EARLY DEVELOPMENT OF SPOROPHYTES

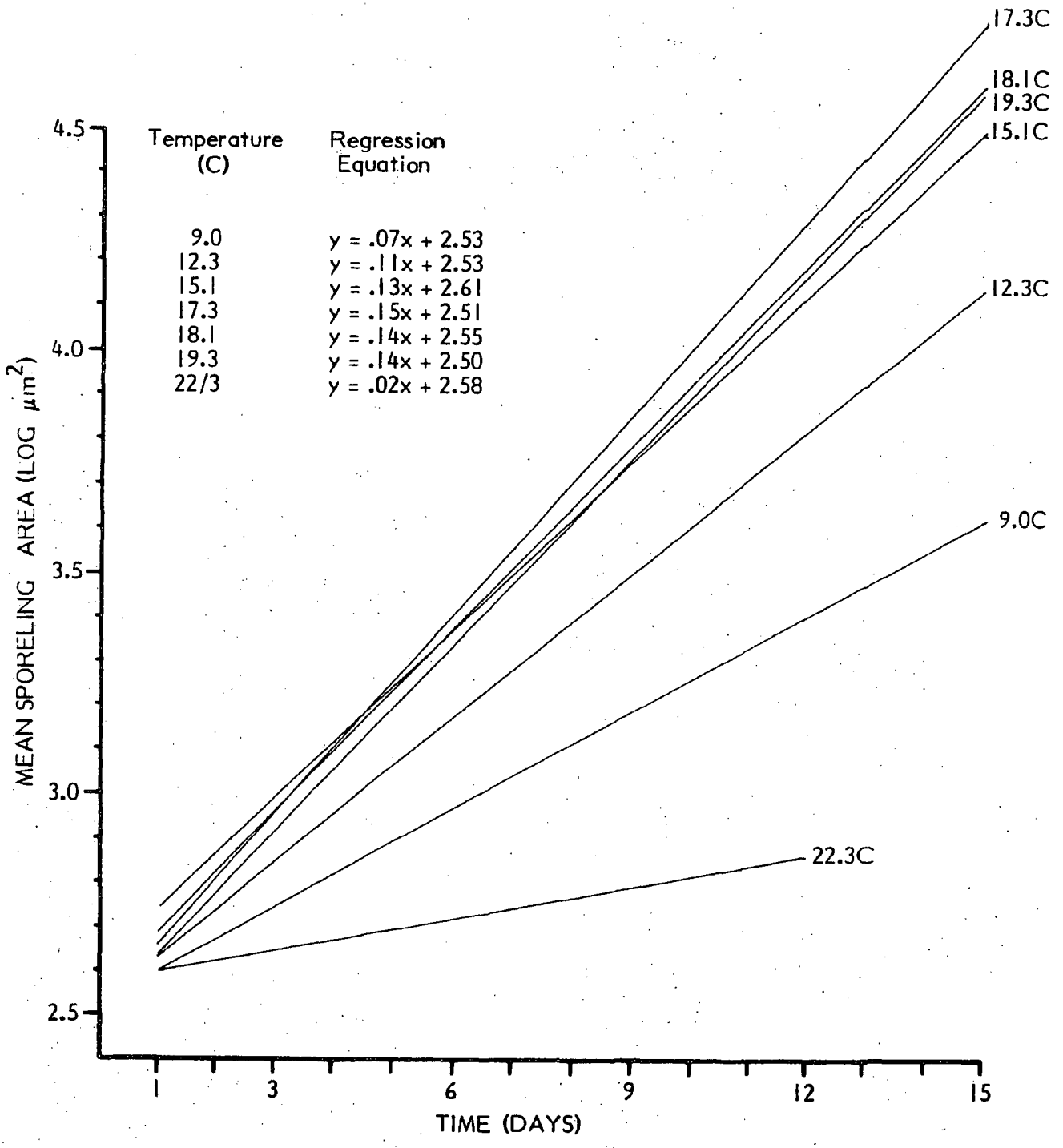


FIGURE 3.9-10

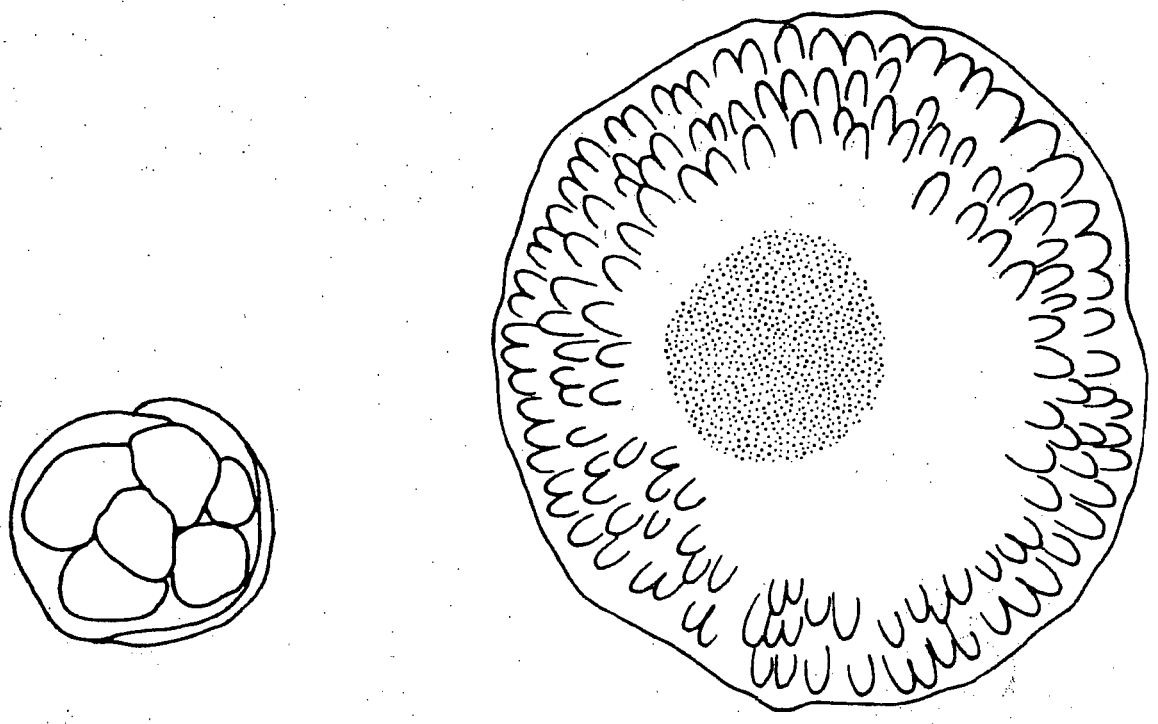
SPORELING DISC AREA VERSUS TIME AT 220 $\mu E/m^2/s$: IRIDESCENT SEAWEED OPTIMUM TEMPERATURE FOR EARLY DEVELOPMENT OF SPOROPHYTES

TABLE 3.9-6

LOG$_{10}$ OF MEAN SPORELING DISC AREA (μm^2)
AT DAYS 1, 3, 6, 9, 12 AND 15:
IRIDESCENT SEAWEED OPTIMUM TEMPERATURE FOR
EARLY DEVELOPMENT OF SPOROPHYTES

Cultures in which erect blade initiation was observed are indicated by "*".

Temperature (C)	Days					
	1	3	6	9	12	15
Light Intensity: 110 $\mu E/m^2/s$						
9.0	2.67	2.74	2.93	3.19	3.44	3.66
12.3	2.62	2.74	3.17	3.58	3.93	4.23*
15.1	2.63	2.88	3.34	3.82	4.15	4.36*
17.3	2.65	2.92	3.48	3.86	4.13*	4.33*
18.1	2.58	2.94	3.37	3.80	4.05	4.34*
19.3	2.53	2.87	3.26	3.66	3.93	4.17*
22.3	2.52	2.67	2.78	--	2.92	--
Light Intensity: 165 $\mu E/m^2/s$						
9.0	2.63	2.71	2.95	3.23	3.43	3.66
12.3	2.61	2.76	3.19	3.58	3.94	4.16*
15.1	2.60	2.73	3.38	3.85	4.20*	4.54*
17.3	2.59	2.84	3.46	3.94	4.28*	4.59*
18.1	2.61	2.91	3.40	3.72	4.08	4.32*
19.3	2.59	2.83	3.39	3.86	4.19	4.42*
22.3	2.54	2.62	2.73	--	2.79	--
Light Intensity: 220 $\mu E/m^2/s$						
9.0	2.63	2.74	2.98	3.18	3.42	3.62
12.3	2.64	2.82	3.21	3.52	3.88	4.10*
15.1	2.67	2.90	3.42	3.89	4.18*	4.37*
17.3	2.60	2.91	3.51	3.95	4.32*	4.64*
18.1	2.56	2.93	3.49	3.97	4.27	4.44*
19.3	2.60	2.90	3.37	3.83	4.27	4.49*
22.3	2.56	2.68	2.77	--	2.86	--



22.3 C, 110 $\mu E/m^2/s$

19.3 C, 220 $\mu E/m^2/s$

FIGURE 3.9-11

COMPARISON OF SPORELING DISC DEVELOPMENT
AT 22.3 C AND 19.3 C DAY 12:
IRIDESCENT SEAWEED OPTIMUM TEMPERATURE
FOR EARLY DEVELOPMENT OF SPOROPHYTES

TABLE 3.9-7

ONE-WAY ANALYSIS OF VARIANCE ON LOG_{10} SPORELING DISC AREA AT DAY 15: IRIDESCENT SEAWEED OPTIMUM TEMPERATURE FOR EARLY DEVELOPMENT OF SPOROPHYTES

Significant values at p = 0.1 are indicated by "*".

Light Intensity ($\mu E/m^2/s$)	Source	Degrees of Freedom	Sums of Squares	Mean Sums	f_{CALC}
220	Temperature	5	20.43	4.086	622.1*
	Error	174	1.143	.007	
	Total	179	21.573		
165	Temperature	5	14.921	2.984	97.7*
	Error	174	5.315	.031	
	Total	179			
110	Temperature	5	10.599	2.120	291.2*
	Error	174	1.267	.007	
	Total	179	11.865		

TABLE 3.9-8

STUDENT-NEWMAN-KEULS (SNK) MULTIPLE RANGE TEST ON LOG_{10} AREA SPORELING DISC AT DAY 15: IRIDESCENT SEAWEED OPTIMUM TEMPERATURE FOR EARLY DEVELOPMENT OF SPOROPHYTES

Means connected by a common line are not statistically different (p = 0.01).

		K =				
		2	3	4	5	6
	Q 01	3.643	4.120	4.403	4.603	4.757
110	LSR ($S\bar{y}$ = .0153)	.056	.063	.067	.070	.073
165	LSR ($S\bar{y}$ = .0321)	.117	.132	.142	.148	.153
220	LSR ($S\bar{y}$ = .0153)	.056	.063	.067	.070	.073

Light Intensity: 110 $\mu E/m^2/s$

Temperature (C) :	15.1	17.3	18.1	19.3	12.3	9.0
Log mean area (μm^2) :	4.36	4.33	4.33	4.23	4.16	3.66

Light Intensity: 165 $\mu E/m^2/s$

Temperature (C) :	17.3	15.1	19.3	18.1	12.3	9.0
Log mean area (μm^2) :	4.58	4.53	4.42	4.32	4.15	3.73

Light Intensity: 220 $\mu E/m^2/s$

Temperature (C) :	17.3	19.3	18.1	15.1	12.3	9.0
Log mean area (μm^2) :	4.64	4.48	4.44	4.36	4.09	3.62

At the lowest light intensity (110 $\mu E/m^2/s$), maximum areal growth occurred at 15.1, 17.3 and 18.1 C and was significantly lower at 19.3 C. At the highest light intensity (220 $\mu E/m^2/s$), maximum areal growth occurred at 17.3 C, was significantly lower at 18.1 and 19.3 C and again was significantly lower at 15.1 C. There was no difference in the 15.1 C areal growth values between the 110 and 220 $\mu E/m^2/s$ treatments but the growth value was higher at 165 $\mu E/m^2/s$. Large increases in growth were not observed by increasing light intensities from 165 to 220 $\mu E/m^2/s$, indicating that light saturation had been reached at 165 $\mu E/m^2/s$ under the experimental conditions used. Maximum growth occurred at 17.3 C in this light intensity range.

Erect Blade Initiation

Initiation of erect blades was observed first on day 12 in the 15.1 C treatments (165 and 220 $\mu E/m^2/s$) and in the 17.3 C treatments (at all light intensities). It is notable that these treatments also had the highest growth rates measured as disc area. By day 15, erect blades had been initiated in all treatments except the 9.0 and 22.3 C treatments at all light intensities. The data on blade initiation are plotted in FIGURE 3.9-12. It is apparent from this figure that blade initiation generally occurred when disc area exceeded 12,000 μm^2, with exceptions at 18.1 and 19.1 C on day 12. This temperature effect only represents a delay in initiation as evidenced by the day 15 data which show blade initiation at these temperatures for all light levels.

3.9.2.3 DISCUSSION

The optimum temperature for early development of iridescent seaweed gametophytes in culture is 17.3 C (63.1 F) when measured as total sporeling disc area after 15 days. At the highest light intensity, growth was significantly lower at 15.1 C (59.2 F) and 18.1 C (64.6 F) than it was at 17.3 C (63.1 F). Very low rates of growth and abnormal development were observed at 22.3 C (72.1 F), indicating that this temperature is at or near the upper limit for normal development of iridescent seaweed sporophyte sporelings. The range of light intensities used in the experiment had a minimal effect on growth. Light saturation may have occurred at 165 $\mu E/m^2/s$.

Total chlorophyll for initial and 60 day plants was evaluated by grinding the dried plants in 90 percent acetone. Absorption values were read on a spectrophotometer at wavelengths specified by Arnon (1949).

The seawater batches for making the PES culture medium used in the experiment were analysed for nitrate-nitrogen and ortho phosphate in order to determine if significant nutrient variations could affect the results of the experiment. These data are given in TABLE 2.9-2 (see SECTION 2.9). Expressed as final concentrations, the enrichment solution added 4,600 $\mu g/l$ N-NO_3 and 330 $\mu g/l$ P-PO_4. Because the medium was changed once per week for the first two weeks and every 3-5 days thereafter, it is not considered likely that nutrients became limiting during the experiment.

3.9.3.2 RESULTS

The material used in this experiment consisted of juvenile gametophytic blades that were mechanically removed from the basal crusts. Removal resulted in a wound at the base of the blade. Data on wound repair were reported in SECTION 2.9.1.

Quantitative measurements of growth were made by taking wet weights and dry weights of the plants sampled from the dishes at 10 day intervals during the experiment. The means and standard deviations of the wet weights are given in TABLE 3.9-9 and of the dry weights in TABLE 3.9-10. The specific growth rate (R) was calculated from the mean dry weight values by employing the following formula:

$$R = 100 \times \frac{\log_e \frac{W_b}{W_a}}{(T_b \; T_a)}$$

where:
- R = percentage increase in dry weight per day
- W_a = mean dry weight at the beginning of the time interval
- W_b = mean dry weight at the end of the time interval
- T_a = the first day of the time interval
- T_b = the last day of the time interval

The specific growth rate values are shown in TABLE 3.9-11.

TABLE 3.9-9

MEAN WET WEIGHT (mg) AND STANDARD DEVIATION (IN PARENTHESES) FOR IRIDESCENT SEAWEED PLANTS SAMPLED FROM EACH DISH THROUGHOUT EXPERIMENT 1a

(N = 10 except where otherwise indicated)

Light Intensity ($\mu E/m^2/s$)	Temperature (C)	DAYS INTO EXPERIMENT 10	20	30	40	51	60
272	22	0.39 (0.14)	0.42 (0.12)	0.72 (0.24)[a]	0.66 (0.14)[a]	0.95 (0.43)[a]	1.28 (0.30)[b]
148	22	0.28 (0.05)	0.34 (0.14)	0.40 (0.15)	0.55 (0.18)	0.70 (0.28)[c]	0.81 (0.36)[b]
25	22	0.25 (0.09)	0.40 (0.16)	0.34 (0.13)	0.46 (0.12)	0.37 (0.07)[a]	0.22 (0.09)[d]
211	19	0.66 (0.30)	2.42 (0.97)	5.96 (1.61)	14.49 (6.91)	22.22 (8.52)	27.52 (5.65)
87	19	0.56 (0.19)	0.92 (0.29)	1.48 (0.26)	3.96 (1.76)	3.99 (1.66)	8.50 (3.79)
272	16	0.91 (0.29)	3.70 (1.02)	11.58 (3.71)	24.20 (5.49)	46.16(17.62)	75.52(21.27)
148	16	0.62 (0.22)	2.20 (0.70)	6.30 (3.24)	13.42 (5.52)	17.23 (7.70)	35.31(16.29)
148	16	0.70 (0.34)	2.48 (0.90)	6.01 (1.62)	11.58 (3.96)	26.83 (7.86)	39.70(15.40)
148	16	0.74 (0.39)	2.33 (0.79)	5.38 (1.89)	13.06 (4.97)	20.17(11.23)	30.05(10.82)
25	16	0.31 (0.13)	0.49 (0.27)	0.44 (0.27)	0.89 (0.37)	0.81 (0.24)	1.03 (0.26)
211	13	0.56 (0.18)	2.35 (1.05)	7.25 (3.79)	12.28 (5.58)	25.53 (7.80)	52.51(22.28)
87	13	0.46 (0.21)	0.90 (0.29)	2.56 (0.67)	4.00 (0.68)	8.84 (4.10)	10.04 (3.99)
272	10	0.35 (0.22)	0.94 (0.32)	2.21 (0.56)	6.59 (3.37)	13.28 (4.09)	21.47 (6.22)
148	10	0.39 (0.19)	1.11 (0.49)	2.31 (1.09)	4.14 (1.30)	11.71 (3.37)	15.36 (3.45)
25	10	0.29 (0.14)	0.35 (0.20)	0.49 (0.18)	0.65 (0.16)	1.19 (0.53)	1.19 (0.29)

Initial weight = 0.30 mg (0.133 S.D.)

[a] N = 6

[b] N = 7

[c] N = 8

[d] N = 9

TABLE 3.9-10

MEAN DRY WEIGHT (mg) AND STANDARD DEVIATION (IN PARENTHESES) FOR IRIDESCENT SEAWEED PLANTS SAMPLED FROM EACH DISH THROUGHOUT EXPERIMENT 1a

(N = 10 except where otherwise indicated)

Light Intensity ($\mu E/m^2/s$)	Temperature (C)	DAYS INTO EXPERIMENT 10	20	30	40	51	60
272	22	0.10 (0.05)	0.08 (0.02)	0.13 (0.04)[a]	0.12 (0.07)[a]	0.16 (0.07)[a]	0.25 0.06)[b]
148	22	0.07 (0.02)	0.06 (0.023)	0.07 (0.03)	0.11 (0.04)	0.12 (0.05)[c]	0.15 (0.09)[b]
25	22	0.05 (0.02)	0.06 (0.02)	0.06 (0.02)	0.08 (0.03)	0.06 (0.01)[a]	0.03 (0.01)[d]
211	19	0.11 (0.04)	0.42 (0.16)	1.09 (0.29)	2.53 (0.84)	3.56 (1115)	4.71 (0.70)
87	19	0.10 (0.04)	0.16 (0.05)	0.28 (0.06)	0.73 (0.29)	0.75 (0.24)	1.53 (0.52)
272	16	0.15 (0.04)	0.58 (0.16)	1.68 (0.48)	4.23 (0.87)	6.33 (1.42)	11.63 (2.48)
148	16	0.12 (0.03)	0.35 (0.11)	0.89 (0.40)	2.18 (0.46)	2.53 (0.97)	5.13 (1.96)
148	16	0.13 (0.07)	0.37 (0.12)	0.87 (0.29)	2.22 (0.75)	3.40 (1.62)	5.12 (1.54)
148	16	0.12 (0.06)	0.38 (0.13)	0.87 (0.17)	2.01 (0.61)	4.01 (0.94)	5.80 (1.74)
25	16	0.06 (0.02)	0.08 (0.04)	0.08 (0.05)	0.17 (0.07)	0.16 (0.04)	0.21 (0.05)
211	13	0.09 (0.02)	0.34 (0.15)	0.95 (0.44)	2.18 (0.93)	3.51 (0.83)	7.48 (2.59)
87	13	0.07 (0.03)	0.14 (0.04)	0.40 (0.10)	0.72 (0.12)	1.42 (0.53)	1.69 (0.44)
272	10	0.07 (0.04)	0.14 (0.06)	0.33 (0.06)	1.19 (0.58)	1.96 (0.53)	3.18 (0.85)
148	10	0.06 (0.03)	0.17 (0.07)	0.36 (0.17)	0.75 (0.24)	1.73 (0.40)	2.33 (0.46)
25	10	0.06 (0.03)	0.06 (0.03)	0.08 (0.03)	0.12 (0.03)	0.21 (0.09)	0.22 (0.05)

[a] N = 6

[b] N = 7

[c] N = 8

[d] N = 9

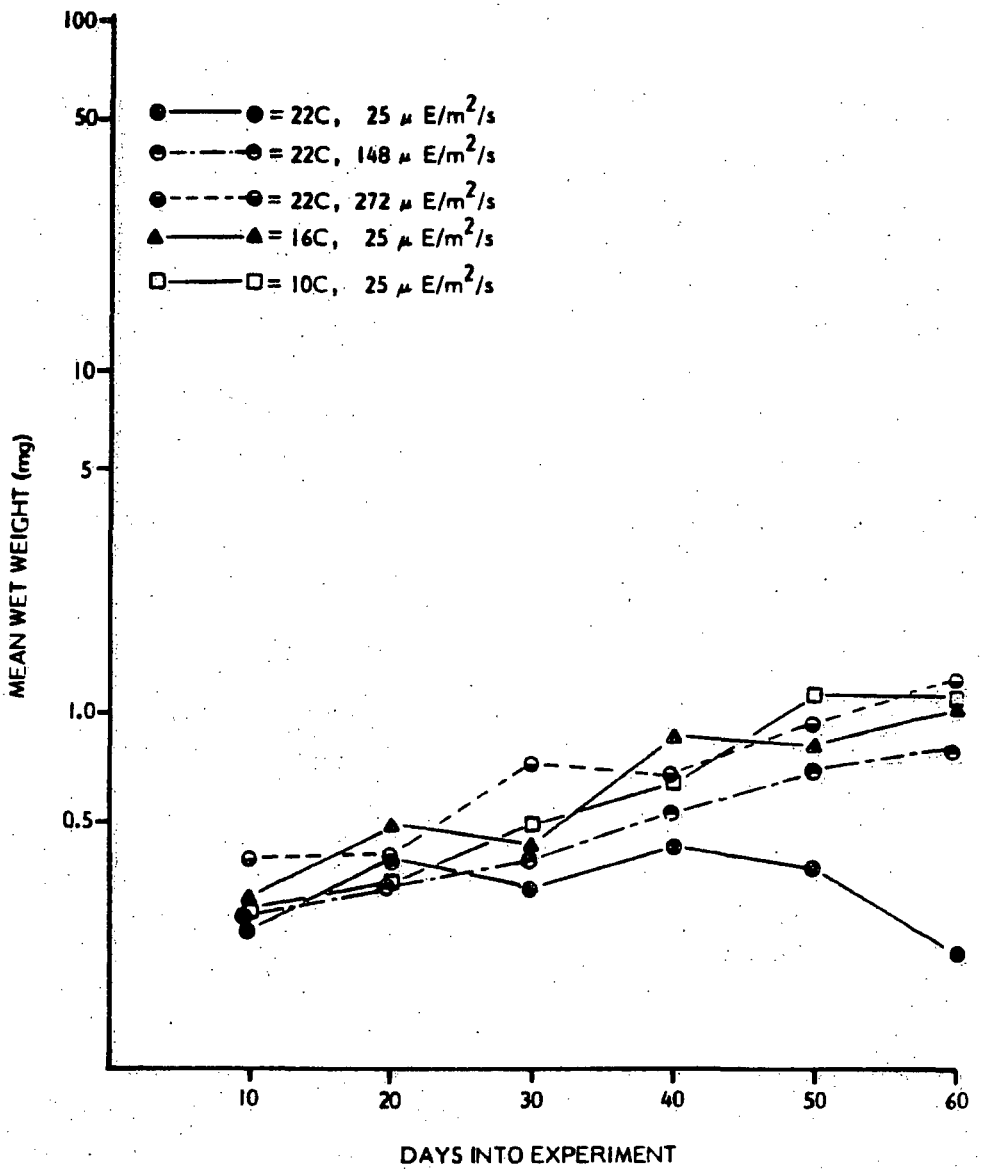


FIGURE 3.9-15

MEAN WET WEIGHT VERSUS TIME OF IRIDESCENT SEAWEED UNDER LOW GROWTH RATE CONDITIONS (EXPERIMENT 1A).

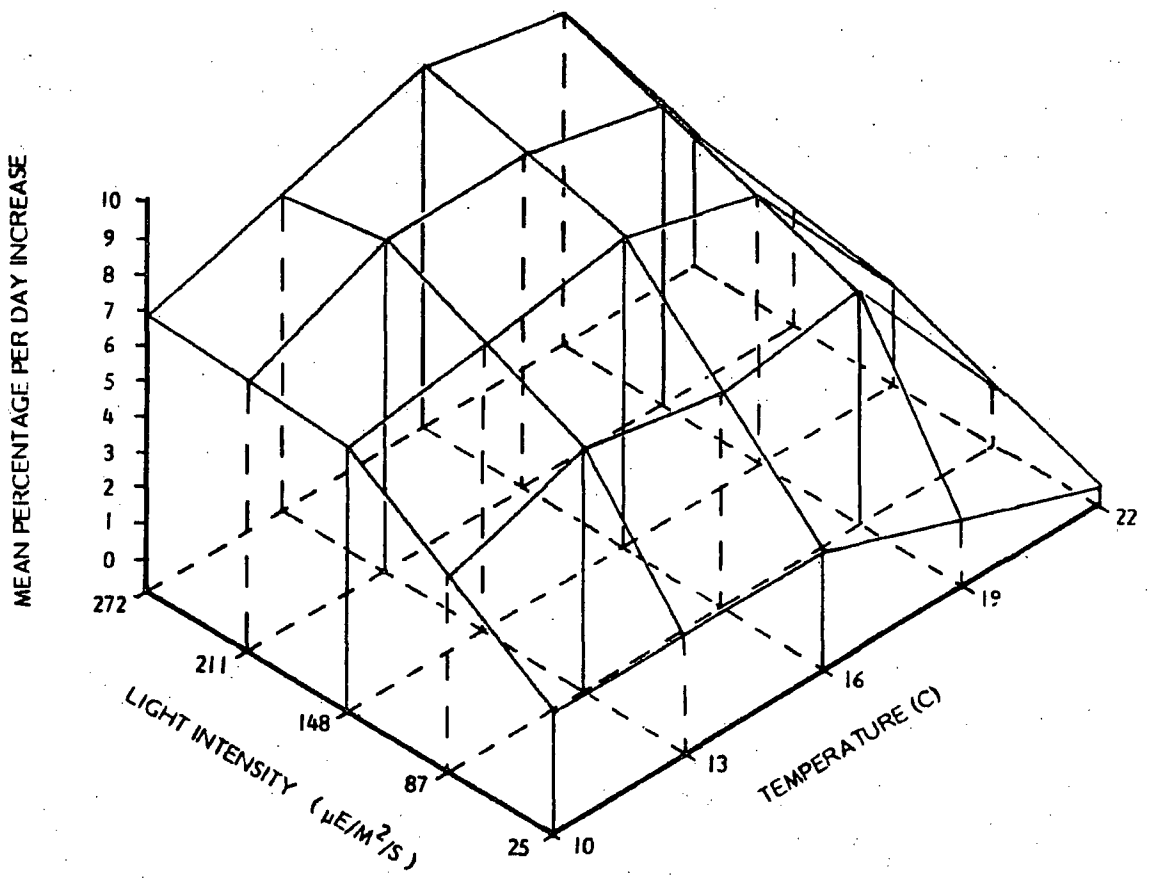


FIGURE 3.9-16

MEAN PERCENTAGE PER DAY INCREASE IN
DRY WEIGHT IRIDESCENT SEAWEED OVER 60 DAYS
(EXPERIMENT 1A).

TABLE 3.9-12

MEAN CHLOROPHYLL CONTENT (AS μg CHLOROPHYLL/mg DRY WEIGHT) IN PLANTS FROM SELECTED TREATMENTS AT DAY 60 IN EXPERIMENT Ia (STANDARD DEVIATION IN PARENTHESES, N = 4)

Chlorophyll content in the blades at the start of the experiment was 1.1 g chlorophyll/mg dry weight (S.D. = 0.11, N = 20)

Temperature (C)	Light Intensity ($\mu E/m^2/s$)	Chlorophyll (μg/mg dry weight)
19	87	2.8(0.3)
16	272	2.4(0.3)
13	87	2.5(0.2)
10	272	1.4(0.2)
10	148	2.0(0.2)

increases as light intensity increased. The effect of light was most marked in the 16C (60.8 F) treatments where the lowest light intensity (25 $\mu E/m^2/s$) was classified in the lowest growth category but growth rates in the higher light intensity treatments (148 and 272 $\mu E/m^2/s$) were among the highest in the experiment. The 10C (50 F) treatments $\mu E/m^2/s$) was classified in the lowest growth category but growth rates in the highest light intensity treatments (148 and 272 $\mu E/m^2/s$) were among the highest in the experiment. The 10C (50 F) treatments showed lower rates. Optimum light intensities for this species under these experimental conditions could not be attained. Under higher light intensities, growth rates could be expected to increase at all temperatures except 10C (50 F). The lethal temperature at 25 $\mu E/m^2/s$ appears to be close to 22C (71.6 F) but at higher light intensities, the lethal limit would appear to be somewhat above 22C (71.6 F)

3.9.4 OPTIMAL TEMPERATURE FOR GROWTH IN FIELD-COLLECTED VERSUS CULTURED PLANTS

Experimental studies of algae in static culture are artifical in that static culture conditions are known to be markedly different from those in nature. It is also known that marine algae may exhibit gross morphological differences compared to naturally occurring plants. One purpose of this experiment was to determine the comparative responses of plants collected from the field and those raised initially in culture, both sets of which were then simulatneously subjected to identical experimental treatments. A second purpose was to determine the responses of the plants through the subadult stage of maximum rates of growth. Thirdly, a comparison would be possible between results of a flow-through system (more comparable to natural conditions) versus those in a static culture (see preceeding section). Finally, an attempt was made to determine whether the two generations in the life history of the species responded differently to the test conditions.

The results of this experiment will be useful in assessing how comparable the responses of plants under controlled conditions are to those which might result in the discharge area. Additional information on more mature stages and on both generations is also provided.

3.9.4.1 METHODS

A summary description of the laboratory methods employed in the investigations into the heat tolerance and optimal temperature for growth of iridescent seaweed comparing cultured to field-collected specimens was presented in SECTION 2.9.2.1. For a more detailed description of the laboratory methods see APPENDIX B (Procedure 303) and protocol see APPENDIX C (experiment 3a).

Algal raceways: The algal raceways were used in this experiment. They were large (15x3x2') insulated tanks with an open flow-through seawater source at one end and a drain at the opposite end. For this experiment the seawater was filtered using a nominal 1 μm filter to reduce contamination. The flow was approximately 2.5 l/s. To increase water circulation, seawater was continually recirculated through spray bars mounted underwater along the sides of the tanks. Timers switched the circulation to all six bars sequentially so that only one bar had water spraying from it at any one time. The plants were attached by plastic clips to a rack on the bottom of the tank. Light was provided by a large bank of overhead fluorescent lights (Vita-Lite) which was controlled by placing neutral density filters over the raceways to obtain lower intensities. Periodic cleaning of the raceways was frequently required primarily due to diatom contamination. This was accomplished by removing the plants and washing the tank with a hot fresh water solution of sodium hypochlorite. The tanks were then thoroughly flushed with fresh and salt water prior to replacement of the plants.

Experimental material: Three types of experimental material were simultaneously exposed to all experimental conditions: field-collected tetrasporophyte, field-collected gametophyte and cultured gametophyte blades. Holdfasts of plants growing in the field which had reproductive blades and juvenile blades were removed from the substratum and brought into the laboratory. Fertilized female (haploid) plants, with characteristic cystocarps could be visually separated from tetrasporangial (diploid) plants. Because male plants could not easily separated by eye, no attempt was made to obtain them; hence, all field-collected gametophytes are females. In the laboratory, small blades ranging from 1.4-2.1 cm in length were detached from the holdfasts and held at 13C.

Gametophyte cultures were grown as described in SECTION 3.9.1.1 until blades ranging from 1.4-2.1 cm in length were obtained. These static cultures were maintained at 13C until the experiment began. These static cultures were maintained at 13C until the experiment began. Cultured gametophytes thus unavoidably consisted both of male and female plants.

Experimental procedure: The combinations of temperature and light intensity used in this experiment are given in TABLE 3.9-13. Measurements of light intensities in all of the treatments were taken prior to the beginning of the experiment and after the experiment was terminated. The mean values of these measurements are given in TABLE 3.9-14. Intensities decreased in all cases and the decreases were greater at the higher light intensities than were those at the lower intensities. These decreases can be attributed to aging of the fluorescent bulbs over the course of the experiment. Plants were introduced into experimental raceways on 3 consecutive days to allow sufficient time for measurements and observations. On day 1, ten individuals of each material type (30 individual total) were measured, weighed, and placed in each of 2 experimental raceways. On day 2, plants were similarly processed and placed in each of 2 additional raceways. On day 3, plants were positioned in the 3 remaining experimental raceways. Each plant was numbered so that the measurements could be related from one time to the next throughout the experiment. Initial blade lengths were measured prior to placement of the plants in the raceways. Initial weights and lengths were obtained from 10 representative plants of the three types, other than those used for the experiment. Subsequently, the plants were freeze-dried and dry weights were measured. Every 10 days for the remainder of the experiment, direct measurements of blade length and wet weight were taken from each plant, all plants were photographed, and observations on mortality and abnormal growth were recorded. Repetitive weighing of blades comparable in size to those used to initiate the experiment (1.4-2.1 cm long) demonstrated that the precision of wet weights at this size is ± 0.01 g. On day 60, following the usual measurements, all plants were freeze-dried and dry weights were measured.

TABLE 3.9-13

MATRIX OF LIGHT INTENSITY AND TEMPERATURE COMBINATIONS USED IN EXPERIMENT 3a.

Tested combinations are denoted by "x"

Light Intensity ($\mu E/m^2/s$)	Temperature (C)						
	9.5	12	ambient[a]	14.5	17	19.5	22
25	x	x	x		x		x
73		x		x	x	x	
21	x		x	x		x	x

[a] Mean = 13C

TABLE 3.9-14

MEAN LIGHT INTENSITY ($\mu E/m^2/s$) BEFORE (B) AND AFTER (A) EXPERIMENT 3a IN ALL TREATMENTS

	25		73		121	
	B	A	B	A	B	A
9.5C	24.8	23.8			120.3	114.6
12C	24.3	22.7	73.6	71.8		
ambient (13.6C)	26.3	25.3			124.3	115.4
14.5C			73.8	68.8	122.7	115.4
17C	26.1	25.7	75.9	73.6		
19.5C			74.3	67.1	119.4	117.8
22C	24.8	23.6			117.6	115.2

On day 4 and every 10 days thereafter, measurements of physical parameters (salinity, pH, dissolved oxygen) were made and water samples were taken for nutrient analysis. Salinity remained at 34 ppt throughout the experiment. The range of pH measured was 6.55-8.45 and dissolved oxygen ranged from 7.0-89 ppm. Seawater samples were analysed for nutrients (nitrate nitrogen and orthophosphate) at the same times as the physical parameters. The mean values for these measurements are given in TABLE 3.9-15. Nitrate nitrogen occurred at markedly lower concentrations on 8/10 and 9/15 and at an intermediate concentration on 8/20. Orthophosphate concentrations generally did not show as much variation and did not correlate with changes in nitrate values. The seawater incoming to all of the tanks comes from the same source so that any variations in nutrients occurred simultaneously in all treatments.

3.9.4.2 RESULTS

The biological data collected in this experiment were of the following types: wet weights, lengths and photographs showing gross morphology were obtained at all sampling times. Dry weight measurements were made at days 0 and 60 only. The field-collected tetrasporophytes that were initially placed into the experiment were accidentally contaminated, probably with an acid, and exhibited high mortality under all conditions (see discussion of mortality below). Therefore, the field-colleced tetrasporophytes were discarded after the 30 day measurements and a new set of field-collected tetrasporophytes was placed into the experimental tanks. These plants were maintained for 25 days at which time (60 days for the other plants) the entire experiment was terminated. For these reasons, the growth data on field-collected tetrasporophytes is not considered to be sufficiently complete to draw conclusions at this time. A more refined analysis of the data will be undertaken at a later time to determine whether limited conclusions about tetrasporophyte growth can be made. For this report, results will be limited to a comparison of the cultured versus the field-collected gametophytes. Mortality data for the four group of plants used in this study were presented in SECTION 2.9.2.

TABLE 3.9-15

MEAN VALUES (STANDARD DEVIATION IN PARENTHESES) OF NITRATE NITROGEN AND ORTHOPHOSPHATE IN SEAWATER AT INTERVALS DURING EXPERIMENT 3a (N = 7)

Data	NO_3-N (μg/l)	PO_4-P (μg/l)
7/21/78	215(52)	30(6)
7/31/78	195(12)	41(9)
8/10/78	46(11)	30(8)
8/20/78	132(25)	29(7)
8/30/78	210(22)	33(10)
9/09/78	201(59)	43(20)
9/15/78	65(31)	27(8)

Significant differences in morphology of field-collected versus cultured gametophytes were apparent in most treatments by day 60. These differences are very difficult to describe and defy quantification in Iridaea. Consequently, a subjective discussion together with examples of the outlines of plants from representative treatments (FIGURES 3.9-17 to 3.9-24) is presented here. In general, the field-collected plants exhibit much less variation in overall size, especially toward smaller size, than do the cultured gametophytes. The outline of field plants is typically more uniform except that the apical portions are often divided into 2 or 3 wedge-shaped tips. The cultured plants also exhibited a greater tendency for growth in three dimensions whereas field-collected plants were more blade-like. Perhaps the greatest similarity between the two types of plants was shown in the 9.5C, 25 $\mu E/m^2/s$ treatment (FIGURES 3.9-17 and 3.9-18). Relative effects of either temperature or light are difficult to extract by examination of plants from a range of temperatures and light intensities (FIGURES 3.9-19 to 3.9-24). These differences may result from two causes: (1) because the cultured groups probably consisted of male and female plants in about a 1:1 ratio whereas the field-collected plants are all females, some morphological variability could be attributed to sex-related genotypes, and (2) rearing under culture conditions may induce a qualitative morphogenetic shift in development in blades at initiation which is not influenced by subsequent environmental conditions.

Mean values of wet weights obtained during the experiment are presented for the cultured gametophytes in TABLE 3.9-16, for the field-collected gametophytes in TABLE 3.9-17 and for the second population of field-collected tetrasporophytes in TABLE 3.9-18. Mean values of dry weights taken at day 60 are presented for the cultured gametophytes in TABLE 3.9-19 and for the field-collected gametophytes in TABLE 3.9-20. Mean lengths of the plants throughout the course of the experiment are presented in TABLE 3.9-21 for cultured gametophytes, TABLE 3.9-22 for the field-collected gametophytes and in TABLE 3.9-23 for the second population of field-collected tetrasporophytes. The wet weight values are a better measure of growth in this plant than are the length measurements and because they were taken throughout the experiment, in contrast to the dry

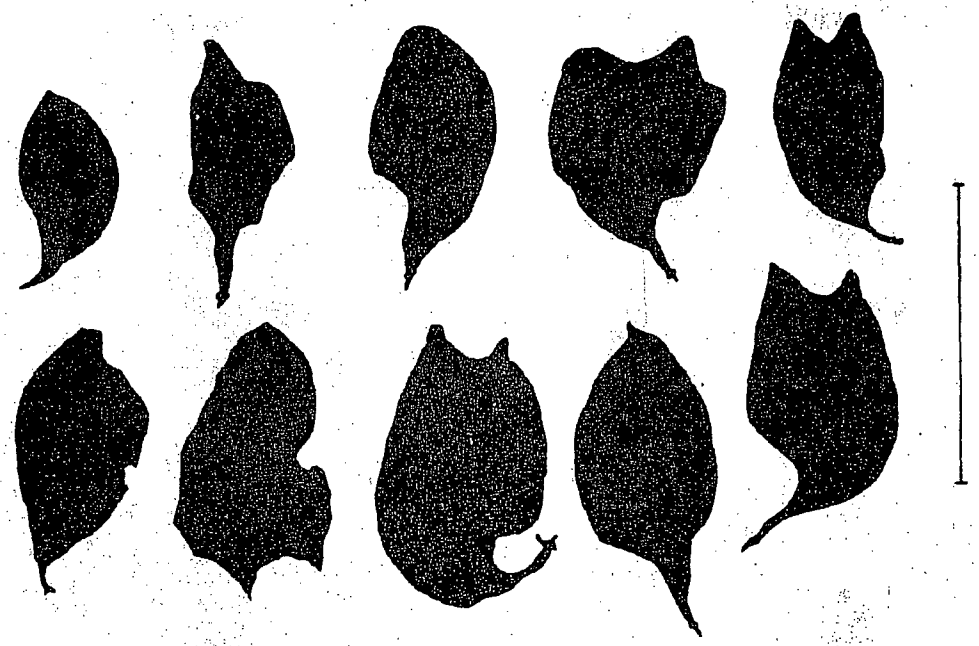


FIGURE 3.9-17

FIELD-COLLECTED GAMETOPHYTES AT LOW TEMPERATURE (9.5C) AND LOW LIGHT INTENSITY ($25\mu Em^{-2}s^{-1}$) AFTER 60 DAYS GROWTH. SCALE: 10cm

FIGURE 3.9-18

CULTURED GAMETOPHYTES AT LOW TEMPERATURE (9.5C) AND LOW LIGHT INTENSITY ($25\mu Em^{-2}s^{-1}$) AFTER 60 DAYS GROWTH. SCALE: 10 cm

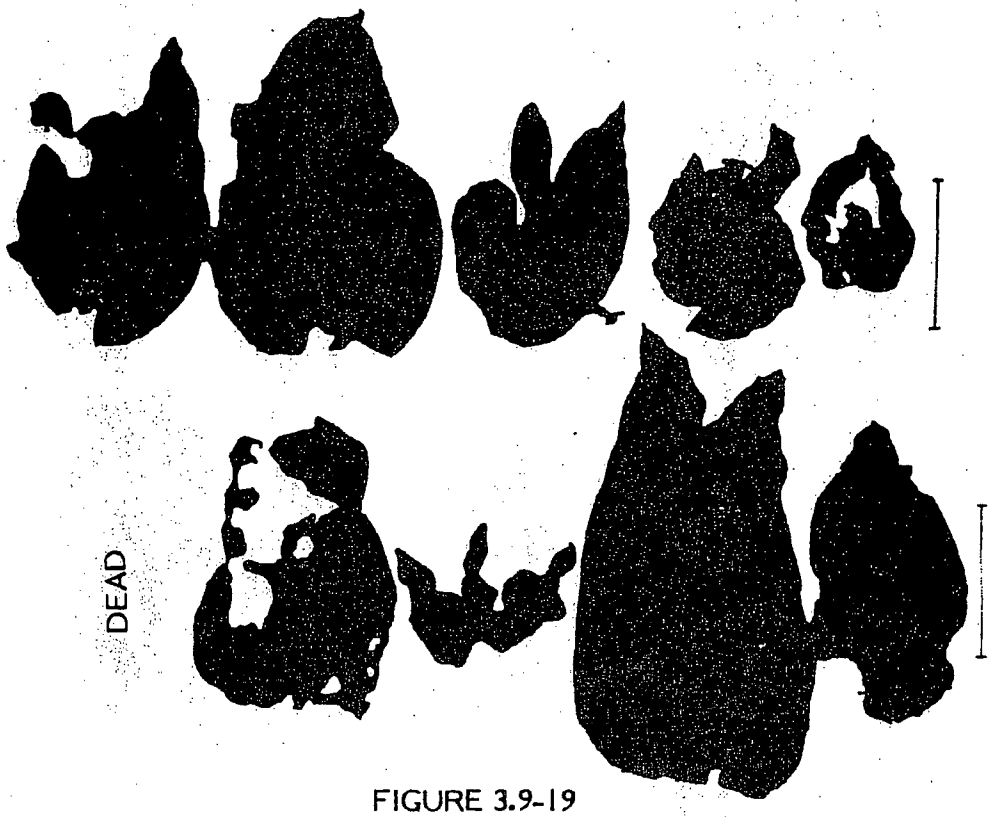


FIGURE 3.9-19

FIELD-COLLECTED GAMETOPHYTES AT MEDIUM TEMPERATURE (14.5C) AND MEDIUM LIGHT INTENSITY ($73\mu Em^{-2}s^{-1}$) AFTER 60 DAYS GROWTH. SCALE: 10 cm

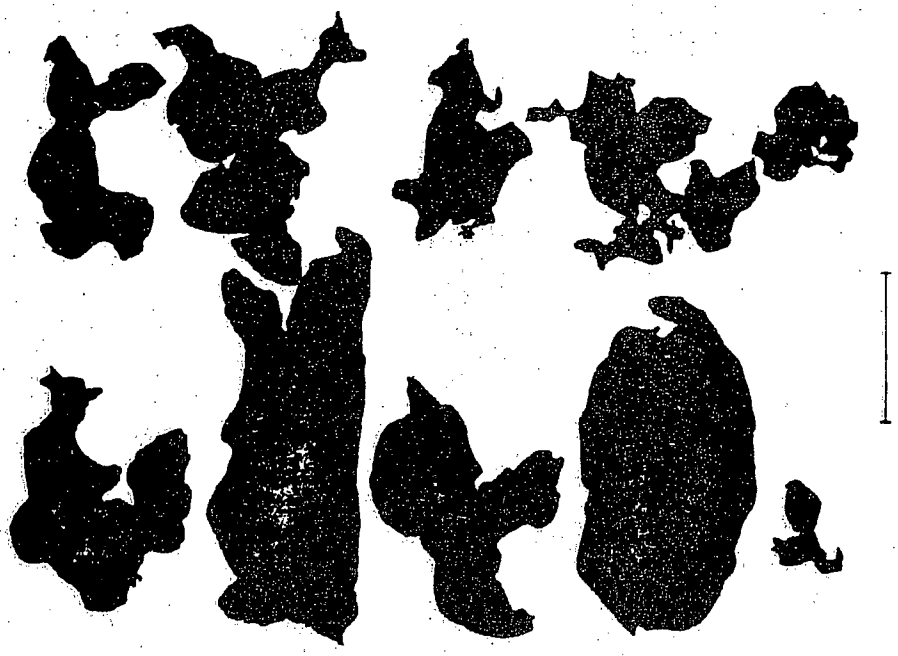


FIGURE 3.9-20

CULTURED GAMETOPHYTES AT MEDIUM TEMPERATURE (14.5C) AND MEDIUM LIGHT INTENSITY ($73\mu Em^{-2}s^{-1}$) AFTER 60 DAYS GROWTH SCALE: 10 cm

FIGURE 3.9-21

FIELD-COLLECTED GAMETOPHYTES AT HIGH TEMPERATURE (19.5C) AND HIGH LIGHT INTENSITY ($121 \mu Em^{-2}s^{-1}$) AFTER 60 DAYS GROWTH. SCALE: 10 cm

FIGURE 3.9-22

CULTURED GAMETOPHYTES AT HIGH TEMPERATURE (19.5C) AND HIGH LIGHT INTENSITY ($121 \mu Em^{-2}s^{-1}$) AFTER 60 DAYS GROWTH. SCALE: 10 cm

FIGURE 3.9-23

FIELD-COLLECTED GAMETOPHYTES AT LOW TEMPERATURE (9.5C) AND HIGH LIGHT INTENSITY ($121 \mu Em^{-2}s^{-1}$) AFTER 60 DAYS GROWTH
SCALE: 10 cm

FIGURE 3.9-24

CULTURED GAMETOPHYTES AT LOW TEMPERATURE (9.5C) AND HIGH INTENSITY ($121 \mu Em^{-2}s^{-1}$) AFTER 60 DAYS GROWTH.
SCALE: 10 cm

TABLE 3.9-16

MEAN WET WEIGHT IN GRAMS AND STANDARD DEVIATION (IN PARENTHESES) FOR IRIDESCENT SEAWEED CULTURED GAMETOPHYTES IN EXPERIMENT 3a.

A dash (--) denotes total mortality.

Temperature (C)	Light Intensity ($\mu E/m^2/s$)	Days into Experiment 0	10	20	30	40	50	60
22	121	0.02(0.01)	0.03(0.01)	0.04(0.01)	0.04(0.01)	0.04(0.01)	0.03	---
22	25	0.02(0.01)	0.03(0.01)	0.03(0.01)	0.04(0.02)	0.05(0.02)	0.04(0.01)	0.04(0.01)
19.5	121	0.02(0.01)	0.06(0.04)	0.17(0.10)	0.38(0.33)	0.78(0.89)	1.95(2.39)	4.20(5.22)
19.5	73	0.02(0.00)	0.05(0.01)	0.11(0.06)	0.28(0.16)	0.60(0.45)	1.35(1.24)	2.91(3.01)
17	73	0.03(0.01)	0.05(0.02)	0.14(0.07)	0.38(0.24)	0.94(0.67)	2.64(2.07)	6.83(5.13)
17	25	0.02(0.01)	0.03(0.01)	0.04(0.02)	0.07(0.03)	0.11(0.05)	0.07(0.04)	0.10(0.07)
14.5	121	0.02(0.01)	0.05(0.02)	0.17(0.07)	0.37(0.20)	0.82(0.54)	3.11(2.35)	18.10(10.47)
14.5	73	0.03(0.01)	0.06(0.02)	0.15(0.07)	0.33(0.17)	0.69(0.35)	2.35(1.28)	6.84(3.70)
12	73	0.03(0.01)	0.05(0.01)	0.13(0.06)	0.34(0.23)	1.73(0.88)	4.59(2.56)	10.52(5.75)
12	25	0.03(0.01)	0.05(0.02)	0.07(0.03)	0.13(0.07)	0.20(0.11)	0.37(0.22)	0.66(0.42)
9.5	121	0.03(0.01)	0.04(0.02)	0.10(0.05)	0.28(0.18)	0.75(0.61)	2.24(1.90)	5.48(3.77)
9.5	25	0.03(0.01)	0.04(0.01)	0.14(0.05)	0.12(0.04)	0.21(0.09)	0.42(0.21)	0.81(0.47)
ambient[a]	121	0.03(0.01)	0.06(0.03)	0.19(0.13)	0.68(0.53)	2.31(1.94)	8.16(6.31)	20.18(12.67)
ambient[a]	25	0.02(0.01)	0.04(0.01)	0.05(0.02)	0.09(0.04)	0.16(0.08)	0.99(0.37)	2.00(0.86)

[a] Mean = 13.6C.

TABLE 3.9-17

MEAN WET WEIGHT IN GRAMS AND STANDARD DEVIATION (IN PARENTHESES) FOR IRIDESCENT SEAWEED FIELD-COLLECTED GAMETOPHYTES IN EXPERIMENT 3a.

A dash (--) denotes total mortality.

Temperature (C)	Light Intensity ($\mu E/m^2/s$)	Days into Experiment 0	10	20	30	40	50	60
22	121	0.04(0.01)	0.07(0.02)	0.06(0.03)	0.06	--	--	--
22	25	0.05(0.01)	0.06(0.01)	0.06(0.03)	0.06(0.01)	0.02(0.01)	--	--
19.5	121	0.05(0.02)	0.13(0.06)	0.31(0.17)	0.78(0.77)	1.87(2.17)	4.99(5.71)	10.54(14.20)
19.5	73	0.04(0.01)	0.11(0.26)	0.23(0.07)	0.47(0.21)	0.73(0.55)	1.36(1.55)	2.57(3.74)
17	73	0.04(0.01)	0.12(0.02)	0.34(0.09)	0.87(0.32)	1.94(0.89)	4.56(2.99)	10.82(7.49)
17	25	0.04(0.01)	0.06(0.20)	0.10(0.04)	0.17(0.07)	0.27(0.14)	0.31(0.20)	0.57(0.39)
14.5	121	0.04(0.01)	0.10(0.03)	0.32(0.14)	0.78(0.44)	1.94(1.26)	6.80(4.40)	18.10(10.47)
14.5	73	0.05(0.10)	0.11(0.02)	0.32(0.10)	0.68(0.29)	1.26(0.71)	3.72(1.90)	9.51(5.15)
12	73	0.05(0.01)	0.12(0.02)	0.29(0.06)	0.69(0.26)	1.73(0.88)	4.59(2.56)	10.52(5.75)
12	25	0.04(0.02)	0.06(0.02)	0.10(0.03)	0.16(0.05)	0.24(0.08)	0.44(0.20)	0.79(0.43)
9.5	121	0.04(0.01)	0.08(0.03)	0.21(0.06)	0.52(0.16)	1.18(0.38)	3.10(1.14)	8.58(2.80)
9.5	25	0.05(0.01)	0.07(0.02)	0.14(0.05)	0.25(0.10)	0.42(0.16)	0.76(0.28)	1.33(0.45)
ambient[a]	121	0.05(0.14)	0.11(0.04)	0.36(0.14)	1.10(0.46)	3.12(1.42)	10.84(5.26)	27.44(13.52)
ambient[a]	25	0.05(0.01)	0.09(0.02)	0.14(0.03)	0.28(0.07)	0.48(0.14)	0.99(0.37)	2.00(0.86)

[a] Mean - 13.6C

TABLE 3.9-18

MEAN WET WEIGHT IN GRAMS AND STANDARD DEVIATION (IN PARENTHESES) FOR THE SECOND POPULATION OF IRIDESCENT SEAWEED FIELD-COLLECTED TETRASPOROPHYTES IN EXPERIMENT 3a.

A dash (--) denotes total mortality.

Temperature (C)	Light Intensity ($\mu E/m^2/s$)	Days into Experiment[a] 0	5	15	25
22	121	0.04(0.02)	0.06(0.02)	0.04(0.03)	--
22	25	0.04(0.01)	0.04(0.01)	0.02	--
19.5	121	0.04(0.01)	0.06(0.02)[b]	0.14(0.05)[c]	--
19.5	73	0.04(0.01)	0.05(0.01)[b]	0.13(0.03)[c]	--
17	73	0.04(0.01)	0.05(0.17)[d]	0.10(0.04)[e]	0.16(0.12)[f]
17	25	0.04(0.01)	0.04(0.01)[d]	0.05(0.03)[e]	0.08(0.06)[f]
14.5	121	0.04(0.01)	0.05(0.01)[d]	0.13(0.06)[e]	0.50(0.20)[f]
14.5	73	0.04(0.00)	0.05(0.01)[d]	0.13(0.04)[c]	0.30(0.16)[f]
12	73	0.04(0.01)	0.04(0.01)[b]	0.11(0.03)[c]	0.14(0.03)[g]
12	25	0.04(0.01)	0.05(0.02)	0.07(0.02)[c]	0.12(0.05)[g]
9.5	121	0.04(0.01)	0.06(0.01)	0.14(0.04)	0.36(0.17)
9.5	25	0.04(0.01)	0.05(0.01)	0.07(0.03)	0.12(0.05)
ambient[h]	121	0.04(0.02)	0.06(0.02)[d]	0.17(0.07)	0.49(0.25)
ambient[h]	25	0.04(0.01)	0.05(0.01)[d]	0.08(0.01)[e]	0.10(0.07)[f]

[a] Day 0 for these plants was different from that of the other groups of plants treated in this experiment (see text).

[b] Recorded day 4

[c] Recorded day 14

[d] Recorded day 6

[e] Recorded day 16

[f] Recorded day 26

[g] Recorded day 24

[h] Mean - 13.6C

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TABLE 3.9-19

MEAN DRY WEIGHT IN GRAMS
AND STANDARD DEVIATION (IN PARENTHESES)
FOR IRIDESCENT SEAWEED CULTURED GAMETOPHYTES
AT DAY 60 IN EXPERIMENT 3a.

A dash (--) denotes total mortality.

Temperature (C)	Light Intensity ($\mu E/m^2/s$)	60-Day Dry Weight
22	121	--
22	25	0.01(0.002)
19.5	121	0.86(1.06)
19.5	73	0.58(0.60)
17	73	1.32(0.99)
17	25	0.02(0.02)
14.5	121	3.48(2.24)
14.5	73	1.22(0.69)
12	73	1.13(1.01)
12	25	0.12(0.08)
9.5	121	1.09(0.75)
9.5	25	0.22(0.18)
13.6 (ambient)	121	3.90(2.66)
13.6 (ambient)	25	0.12(0.07)

TABLE 3.9-20

MEAN DRY WEIGHT IN GRAMS AND STANDARD DEVIATION (IN PARENTHESES) FOR IRIDESCENT SEAWEED FIELD-COLLECTED GAMETOPHYTES AT DAY 60 IN EXPERIMENT 3a.

A dash (--) denotes total mortality.

Temperature (C)	Light Intensity ($\mu E/m^2/s$)	60-Day Dry Weight
22	121	--
22	25	--
19.5	121	2.07(2.78)
19.5	73	0.52(0.70)
17	73	2.32(1.53)
17	25	0.12(0.08)
14.5	121	3.62(2.16)
14.5	73	1.95(1.04)
12	73	1.99(1.08)
12	25	0.15(0.08)
9.5	121	1.89(0.67)
9.5	25	0.27(0.11)
13.6 (ambient)	121	5.39(2.68)
13.6 (ambient)	25	0.41(0.17)

TABLE 3.9-21

MEAN LENGTH (mm) AND STANDARD DEVIATION (IN PARENTHESES) FOR IRIDESCENT SEAWEED CULTURED GAMETOPHYTES IN EXPERIMENT 3a.

A dash (--) denotes total mortality.

Temperature (C)	Light Intensity ($\mu E/m^2/s$)	Days Into Experiment						
		0	10	20	30	40	50	60
22	121	17(02)	20(03)	21(04)	21(03)	21(03)	13*	--
22	25	17(02)	17(04)	17(06)	18(06)	22(03)	20(01)	20(02)
19.5	121	17(02)	27(06)	40(10)	35(20)	64(35)	80(51)	110(68)
19.5	73	17(02)	24(04)	33(01)	45(21)	58(29)	76(43)	89(63)
17	73	18(02)	25(05)	40(12)	58(21)	86(35)	127(60)	186(93)
17	25	17(01)	18(04)	24(06)	29(08)	35(12)	24(11)	25(08)
14.5	121	18(02)	26(05)	45(12)	58(20)	79(38)	148(77)	216(126)
14.5	73	17(02)	24(04)	37(09)	50(14)	75(28)	123(47)	186(74)
12	73	18(02)	25(04)	36(12)	56(22)	84(39)	137(66)	197(97)
12	25	19(03)	24(05)	31(08)	39(12)	51(19)	66(26)	82(35)
9.5	121	18(01)	23(05)	34(08)	52(17)	80(29)	129(49)	193(70)
9.5	25	18(02)	23(02)	30(03)	39(05)	53(09)	72(16)	96(26)
ambient[a]	121	18(03)	26(06)	46(14)	74(28)	119(56)	222(102)	323(135)
ambient[a]	25	18(02)	22(04)	26(09)	34(12)	42(16)	60(20)	71(24)

[a] Mean = 13.6C

* No SD - one plant survived

TABLE 3.9-22

MEAN LENGTH (mm) AND STANDARD DEVIATION (IN PARENTHESES)
FOR IRIDESCENT SEAWEED FIELD-COLLECTED GAMETOPHYTES IN EXPERIMENT 3a.

A dash (--) denotes total mortality.

Temperature (C)	Light Intensity ($\mu E/m^2/s$)	Days Into Experiment						
		0	10	20	30	40	50	60
22	121	16(01)	20(04)	18(07)	22*	--	--	--
22	25	16(02)	19(02)	17(08)	22(02)	07(02)	--	--
19.5	121	17(02)	27(04)	39(10)	53(24)	70(46)	100(83)	141(119)
19.5	73	16(02)	26(02)	36(05)	47(12)	60(30)	69(39)	98(69)
17	73	16(01)	27(03)	46(06)	72(13)	108(20)	156(44)	215(63)
17	25	16(02)	23(03)	29(07)	38(08)	47(13)	43(20)	55(31)
14.5	121	17(02)	28(05)	48(15)	73(26)	111(51)	201(80)	296(108)
14.5	73	16(01)	26(03)	44(08)	64(14)	88(34)	149(44)	219(70)
12	73	17(02)	27(03)	43(05)	65(13)	100(27)	156(47)	220(69)
12	25	16(02)	20(02)	27(03)	36(05)	46(10)	60(17)	77(26)
9.5	121	16(02)	23(05)	39(05)	60(09)	92(16)	150(27)	222(39)
9.5	25	16(02)	23(02)	31(04)	42(05)	59(07)	81(10)	104(12)
ambient[a]	121	17(02)	28(04)	52(10)	88(20)	138(40)	240(65)	345(99)
ambient[a]	25	17(02)	24(02)	35(03)	50(06)	66(09)	95(17)	130(23)

[a] Mean = 13.6C

* No SD - one plant survived

TABLE 3.9-23

MEAN LENGTH (mm)
AND STANDARD DEVIATION (IN PARENTHESES)
FOR THE SECOND POPULATION OF IRIDESCENT SEAWEED
FIELD-COLLECTED TETRASPOROPHYTES IN EXPERIMENT 3a.

A dash (--) denotes total mortality.

Temperature (C)	Light Intensity ($\mu E/m^2/s$)	Days Into Experiment[a] 0	5	15	25
22	121	18(02)	19(03)	15(05)	--
22	25	16(02)	16(02)	08(02)	--
19.5	121	19(02)	21(03)[b]	31(06)[c]	37(12)[g]
19.5	73	18(02)	19(02)[b]	29(04)[c]	LOST
17	73	16(03)	19(04)[d]	24(05)[e]	25(13)[f]
17	25	17(03)	18(03)[d]	18(05)[e]	22(10)[f]
14.5	121	17(02)	20(02)[d]	30(08)[e]	56(11)[f]
14.5	73	17(01)	20(01)[d]	33(06)[e]	45(12)[f]
12	73	17(02)	18(02)[b]	29(03)[c]	45(07)[g]
12	25	19(01)	19(02)[b]	24(03)[c]	32(06)[g]
9.5	121	17(02)	19(02)	30(06)	49(14)
9.5	25	17(02)	17(02)	23(05)	31(08)
ambient[h]	121	16(03)	19(03)[d]	33(07)	54(17)
ambient[h]	25	16(02)	18(02)[d]	25(02)[e]	35(05)[f]

[a] Day 0 for these plants was different from that of the other groups of plants tested in this experiment (see text).

[b] recorded day 4

[c] recorded day 14

[d] recorded day 6

[e] recorded day 16

[f] recorded day 26

[g] recorded day 24

[h] Mean = 13.6C

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weights, they have been used for detailed analysis. The data for the tetrasporophytes have been omitted because of the short time over which measurements are available. The log-transformed wet weights for cultured and field-collected gametophytes were regressed against time for each treatment (see TABLES 3.9-24 and 3.9-25). The parameter of major importance is the unstandardized regression coefficient, i.e., slope of the growth line. In order to compare the various treatments, the y-intercept was ignored and the growth lines were plotted with a y-intercept at the origin. These lines are shown for the cultured gametophytes in FIGURE 3.9-25 and for the field-collected gametophytes in FIGURE 3.9-26. A slightly different picture is obtained by plotting the slope versus temperature, as shown in FIGURE 3.9-27. In general, the field-collected gametophytes appear to have responded differently from the cultured gametophytes. The field-collected plants exhibited a greater spread in growth rates, showing both higher and lower rates than the cultured plants. The plants receiving the lowest light intensity (25 $\mu E/m^2/s$) and the highest temperature (22C) had the lowest growth rates. The cultured plants at 19.5C also had low growth rates but the field-collected plants exposed to 19.5C had markedly higher growth rates (FIGURE 3.9-26). It is interesting that the field-collected plants almost without exception had higher growth rates than the cultured plants at the intermediate and high light intensities. At the lowest light intensity, on the contrary, the cultured plants had consistently higher growth rates. In nearly all cases, higher growth rates were obtained at higher light intensities. The highest growth rates occurred in the plants exposed to ambient temperatures which may indicate that constant temperature depresses growth rates in this species. Three-dimensional graphs constructed from the 60-day dry weight values are shown in FIGURE 3.9-28 for cultured plants and in FIGURE 3.9-29 for field-collected plants. These graphs illustrate the fact that a determination of the optimum temperature for growth cannot be made with these data, and that any such determination will probably have to specify light levels. It is evident that saturation light levels were not reached in this experiment. Furthermore, because of the absence of data at 12C and 17C for the highest light level, the optimum temperature for that level was not determined except to say that it lies between 9.5C and 19.5C. The rate of growth appeared to be definitely lower at 19.5C. The lethal temperature for this species thus would appear to be somewhat above 19.5C. Much higher light intensities than it was possible to

TABLE 3.9-24

REGRESSION COEFFICIENTS OF LN WET WEIGHT VERSUS TIME FOR CULTURED GAMETOPHYTES OF IRIDESCENT SEAWEED IN EXPERIMENT 3a.

Temperature (C)	Light Intensity ($\mu E/m^2/s$)	Y-intercept	Slope
9.5	121	-3.34	0.87
9.5	25	-3.15	0.56
13.6 (ambient)	121	-3.31	1.09
13.6 (ambient)	25	-3.01	0.59
14.5	121	-3.24	0.95
14.5	73	-3.00	0.85
12.0	73	-3.06	0.88
12.0	25	-3.27	0.47
17.0	73	-2.95	0.84
17.0	25	-3.21	0.44
19.5	121	-2.79	0.63
19.5	73	-2.79	0.54
22.0	121	-3.44	0.89
22.0	25	-3.33	0.62

TABLE 3.9-25

REGRESSION COEFFICIENTS OF LN WET WEIGHT VERSUS TIME FOR FIELD-COLLECTED GAMETOPHYTES OF IRIDESCENT SEAWEED IN EXPERIMENT 3a.

Temperature (C)	Light Intensity ($\mu E/m^2/s$)	Y-intercept	Slope
9.5	121	-4.03	0.90
9.5	25	-3.73	0.53
12.0	73	-3.82	0.87
12.0	25	-3.60	0.47
13.6 (ambient)	121	-4.19	1.17
13.6 (ambient)	25	-3.87	0.50
14.5	121	-3.88	0.91
14.5	73	-3.74	0.93
17.0	73	-3.84	0.90
17.0	25	-3.79	0.33
19.5	121	-3.51	0.73
19.5	73	-3.63	0.66
22	121	-4.06	0.62
22	25	-3.58	0.50

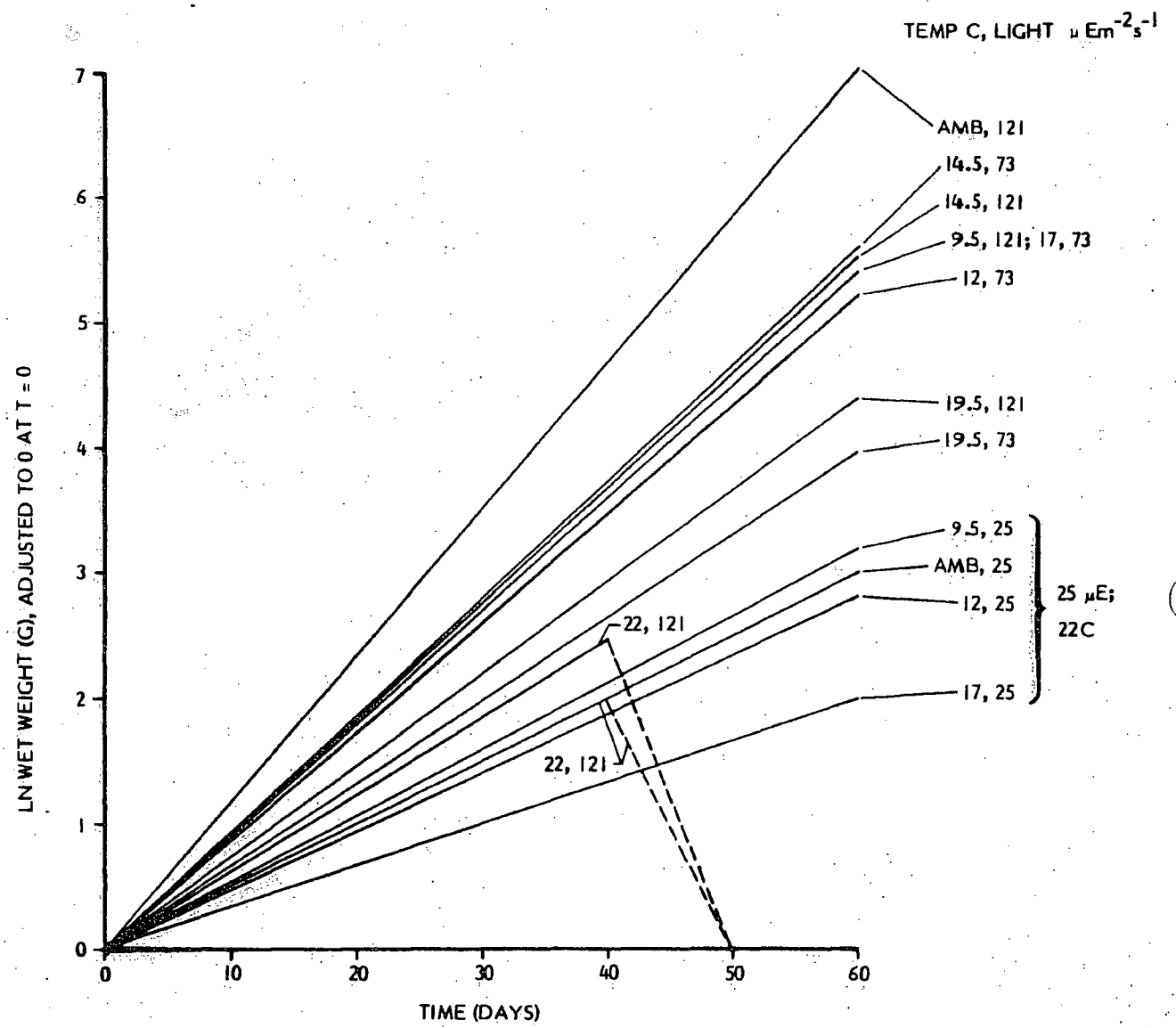


FIGURE 3.9-25

REGRESSION LINES (ADJUSTED TO 0 AT T=0) OF LN WET WEIGHT VERSUS TIME FOR CULTURED GAMETOPHYTES OF IRIDESCENT SEAWEED IN EXPERIMENT 3A.

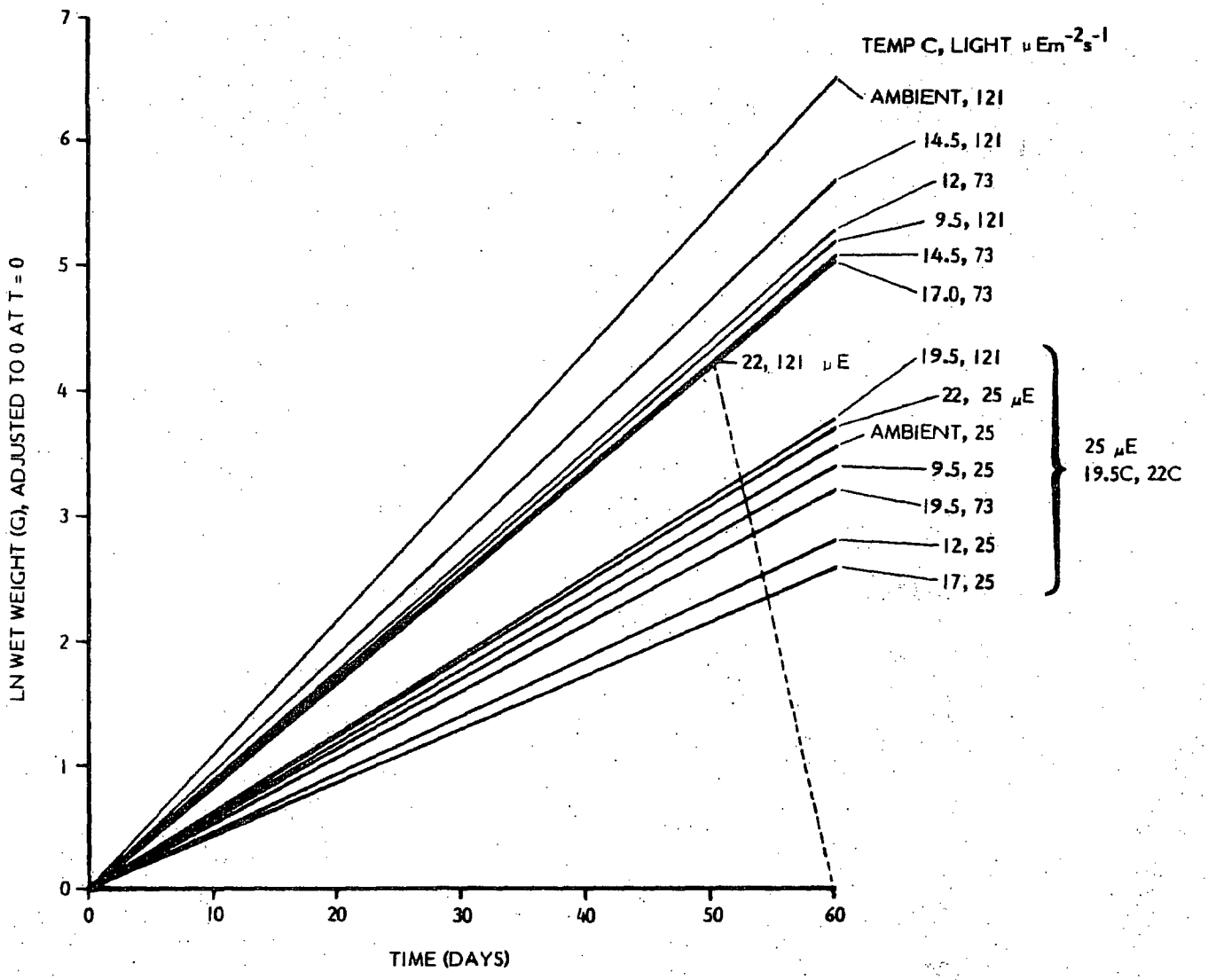


FIGURE 3.9-26

REGRESSION LINES (ADJUSTED TO 0 AT T=0) OF
LN WET WEIGHT VERSUS TIME FOR FIELD-COLLECTED GAMETOPHYTES OF
IRIDESCENT SEAWEED IN EXPERIMENT 3A.

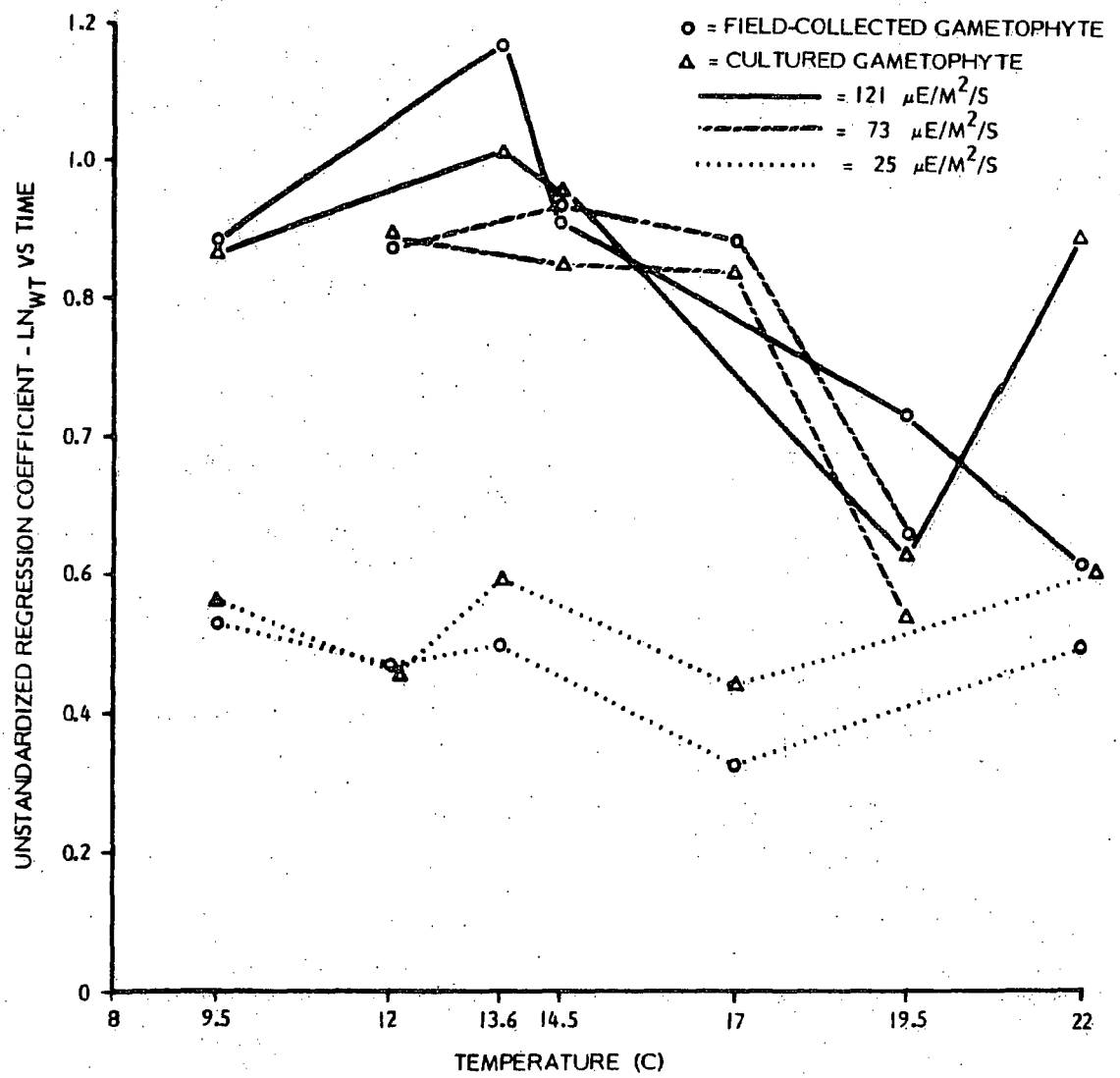


FIGURE 3.9-27

UNSTANDARDIZED REGRESSION COEFFICIENTS OF
LN WET WEIGHT VERSUS TIME (GROWTH RATE) VERSUS TEMPERATURE
OF IRIDESCENT SEAWEED IN EXPERIMENT 3A.

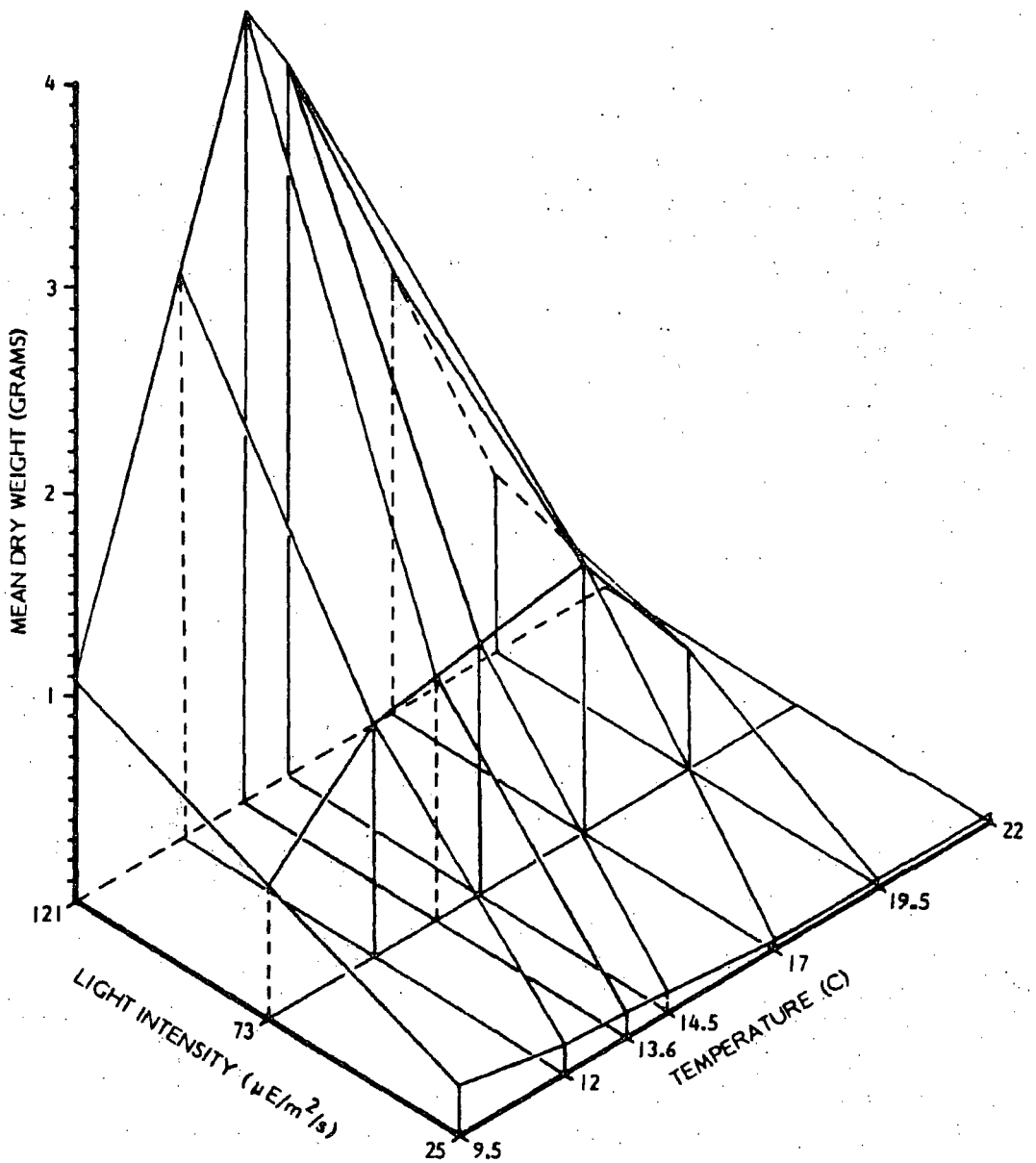


FIGURE 3.9-28

MEAN DRY WEIGHT OF IRIDESCENT SEAWEED CULTURED GAMETOPHYTES AFTER 60 DAYS (EXPERIMENT 3A).

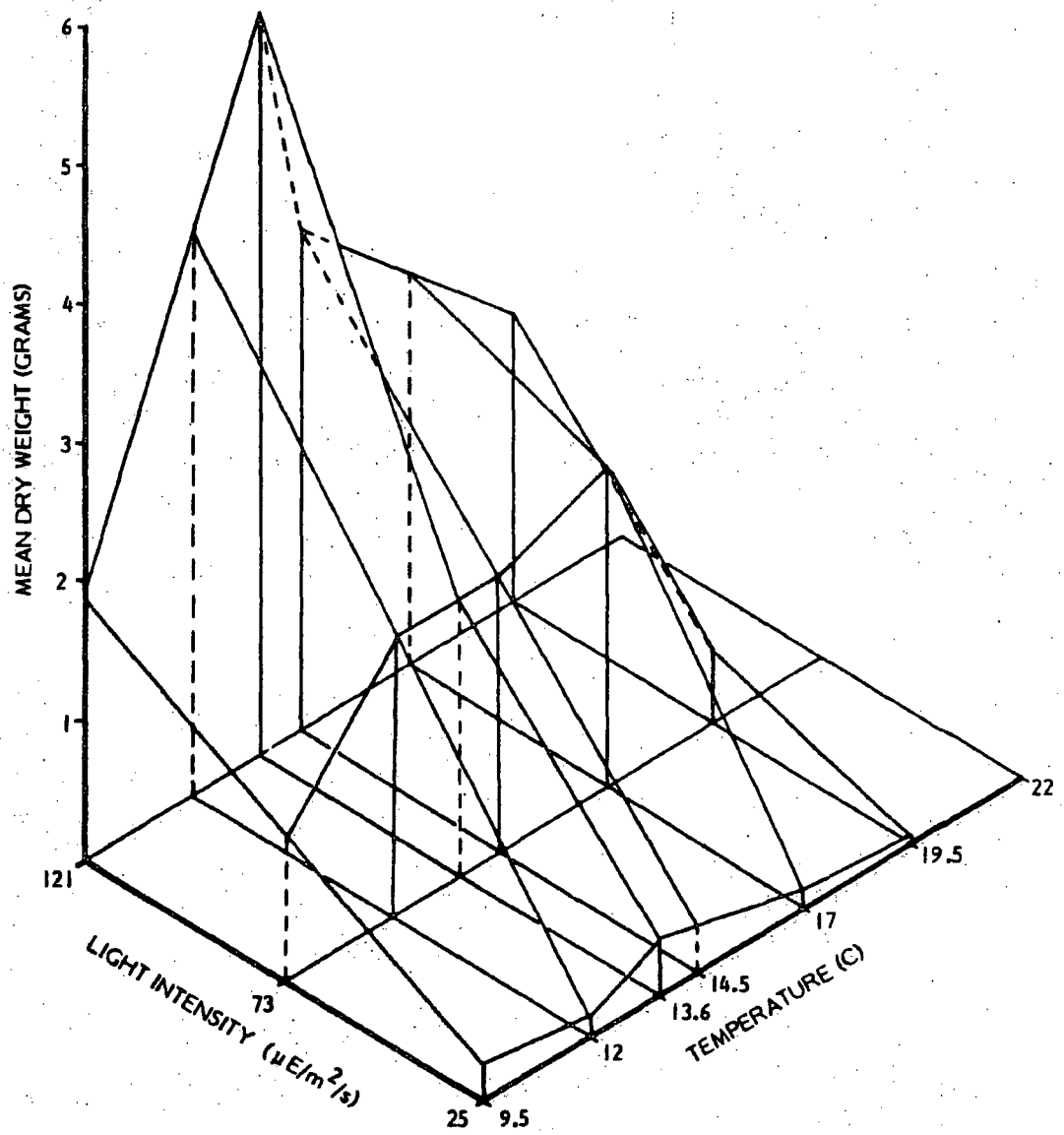


FIGURE 3.9-29

MEAN DRY WEIGHT OF IRIDESCENT SEAWEED
FIELD COLLECTED GAMETOPHYTES AFTER 60 DAYS
(EXPERIMENT 3A).

attain in the laboratory, might extend the lethal temperature somewhat higher by increasing photosynthetic rates to compensate for increased rates of respiration.

3.9.4.3 DISCUSSION

The results of this experiment indicate that cultured plants respond differently to identical experimental conditions than do field-collected plants. A determination of possible differences between generations was not possible at this time due to contamination of some experimental material. Caution must be exercised in applying the results of such experiments to possible effects of the discharge. In general, the relative differences between treatments were the same in both the field and cultured groups of plants. The time course of mortality demonstrated that it is necessary to conduct long-term experiments on plants in order to determine the ultimate response to a set of conditions. Somewhat in contrast to the results of the static culture experiment, the results of this flow-through experiment demonstrate the marked synergistic effect of light intensity. An important observation is the fact that higher light intensities than were obtainable in the laboratory would probably result in a higher temperature tolerance. The maximum light energy supplied in the experimental setup is estimated to be 25 percent of full daylight at low tide and 60 percent of sunlight energy of heavily clouded days. Higher laboratory light intensities (400 percent increase) are not practical, and a higher temperature tolerance will remain theoretically probable.

Lethal temperatures were reached by 60 days at 22C (71.6 F) except for two cultured plants. The optimal temperature for growth could not be well defined by this experiment due to physical constraints. Higher light intensities and fluctuating temperatures would probably be required to realistically define the optimal temperature for growth in this species.

3.10 OTHER SPECIES

In addition to the important species discussed in the foregoing sections, growth or early development of six additional species were studied. These included the black surfperch (Embiotoca jacksoni), two snails (Tegula brunnea and T. funebralis) and three seaweeds (oarweed, Laminaria dentigera, Botryoglossum farlowianum and Iridae cordata). The results of these experiments are presented in the following sections.

3.10.1 BLACK SURFPERCH (EMBIOTOCA JACKSONI) GROWTH

Black surfperch (Embiotoca jackonsi) are very similar in appearance, distribution and behavior to the congeneric striped surfperch (E. lateralis), which is designated as a representative important species for the Diablo Canyon area. Although striped surfperch were the fifth most numerous fish observed on fish transects in and around Diablo Cove, they were not present in large aggregations which could be easily collected in sufficient numbers for experimental purposes. Black surfperch, however, was the sixth most abundant fish observed on the fish transects, and this species was readily obtained in large numbers from nearby Morro Bay.

The study objective of this experiment was to determine that temperature or range of temperatures which produce maximum growth under laboratory conditions. A brief summary of the methods used to determine the optimum temperature for growth of black surfperch is presented below. For a more detailed description of the methods used see APPENDIX B (procedure 320) and protocol see APPENDIX C (experiment 20a).

3.10.1.1 METHODS

Juvenile black surfperch were collected using a 3 m otter trawl in July and August 1978. All fish were taken from the northern end of Morro Bay adjacent to the intakes of the PGandE Morro Bay power plant. The fish were held in the thermal effects laboratory in 4 x 8 ft fiberglass tanks at ambient seawater

temperture until just prior to the commencement of the experiment at which time the temperature of the water was raised or lowered to the prescribed experimental temperatures. Target temperatures for the experimental tanks were 12, 16, 18 and 20 C plus one tank maintained at ambient temperatures. Following an acclimation period, at the experiment temperatures, all thirty fish in a test tank were anesthetized with MS-222 and their standard lengths and wet weights recorded. These initial measurements marked the start of the experiment (elapsed days: 0) for that particular tank.

All test populations were measured between 30 April 1979 and 8 May 1979. During these first measurements, approximately one-third of the fish held at 20 C died as a result of the combined stress of high temperature and handling. These fish were replaced. In order to avoid a re-occurrence of this type of mortality, the fish were not measured midway through the experiment as called for by the procedure. All fish were fed an excess ration of frozen brine shrimp daily between 0900 and 1100 for the duration of the experiment. On days when measurements were made, feeding was witheld until after the data had been collected. Final measurements were made after 59 days. Growth rates were calculated using the formula for exponential growth (Brett, et al. 1969): $w = be^{kt}$ (where w = weight, t = time and b and k are constants). When ln w is plotted against t, the slope k x 100 is equal to the growth rate. Temperature records were recorded hourly throughout the experiment by a computer and the data were transcribed manually for statistical summarization.

3.10.1.2 RESULTS

Temperature and growth data are presented in TABLE 3.10.1-1 Individual weights from each treatment group were plotted on a log scale against time and a regression line was calculated for each treatment group (FIGURE 3.10.1-1). The slope of the regression line multiplied by 100 is equal to the growth rate (percentage increase in weight per day = % ΔW/day). Growth rates were plotted against temperature in FIGURE 3.10.1-2.

TABLE 3.10.1-1

BLACK SURFPERCH OPTIMUM TEMPERATURE FOR GROWTH:
SUMMARY OF TEMPERATURE AND GROWTH DATA

Target Temperature (C)	Ambient	12	16	18	20
Mean Temperature (C)*	12.2 ± 2.8	12.1 ± 0.2	16.1 ± 0.2	18.0 ± 0.3	20.0 ± 0.8
Start Date	04-30-79	05-01-79	05-04-79	05-07-79	05-08-79
Finish Date	06-28-79	06-29-79	07-02-79	07-05-79	07-06-79
Test Period (Elapsed Days)	59	59	59	59	59
No. Fish per treatment (Day 1)	30	30	30	30	30
No. Fish per treatment (Day 60)	30	30	30	28	30
Mean Standard Length (mm) (Day 1)	86 ± 2	79 ± 2	89 ± 2	89 ± 4	84 ± 4
Mean Wet Weight (g) (Day 1)	25.3 ± 2.4	19.7 ± 1.6	24.3 ± 1.6	25.1 ± 3.0	23.4 ± 2.4
Mean Standard Length (mm) Day 60	94 ± 2	90 ± 2	99 ± 2	103 ± 4	98 ± 2
Mean Wet Weight (g) (Day 60)	34.5 ± 3.0	29.2 ± 2.2	38.7 ± 2.8	45.5 ± 5.2	38.1 ± 3.4
Growth Rate (% Δ W/day)	.54	.67	.78	1.03	.84

*Temperature data are ± 2 SD all other data are ± 2 SE

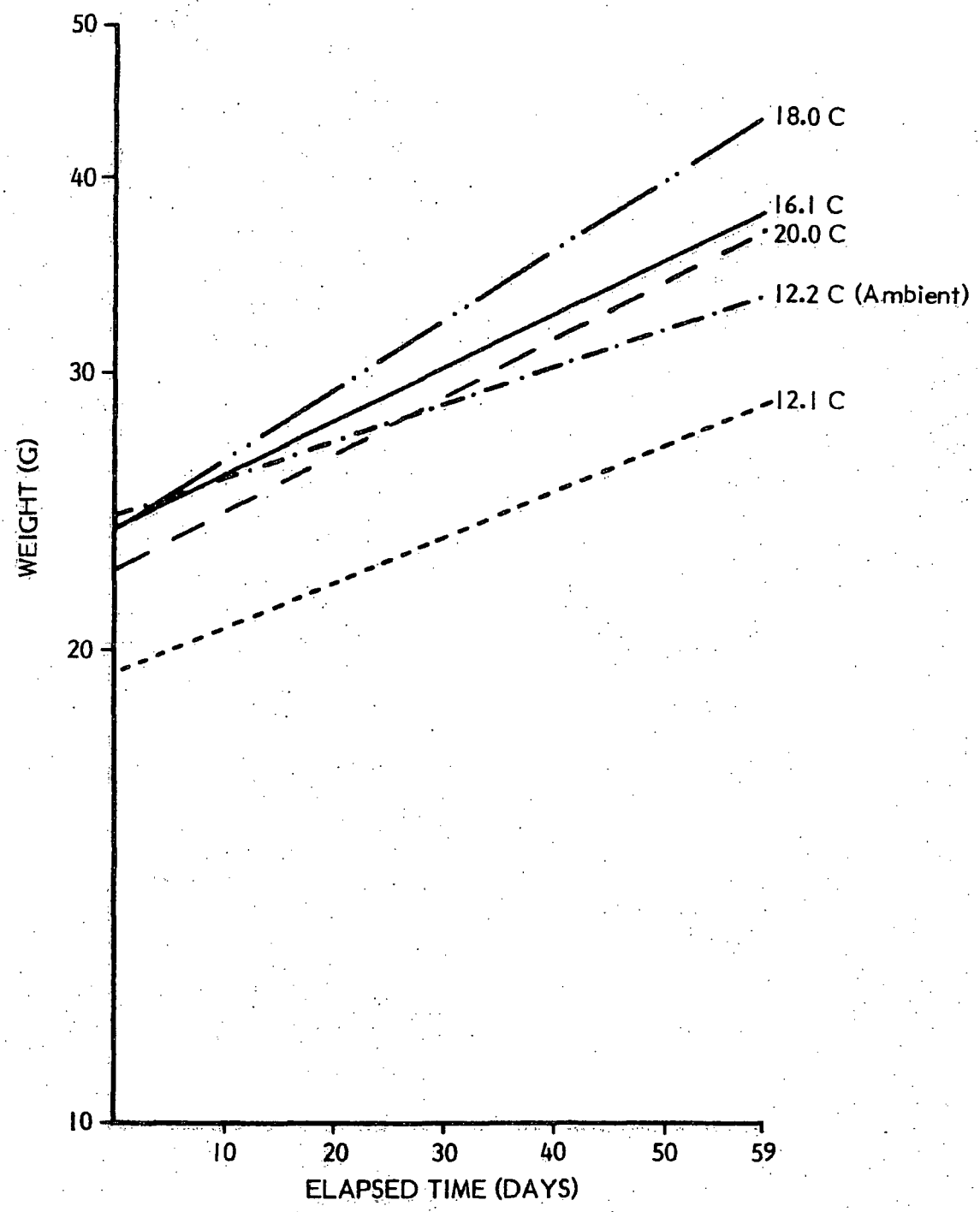


FIGURE 3.10.1-1

BLACK SURFPERCH OPTIMUM TEMPERATURE FOR GROWTH:
REGRESSION LINES OF TOTAL WEIGHT VERSUS
TIME AT FIVE TEMPERATURES

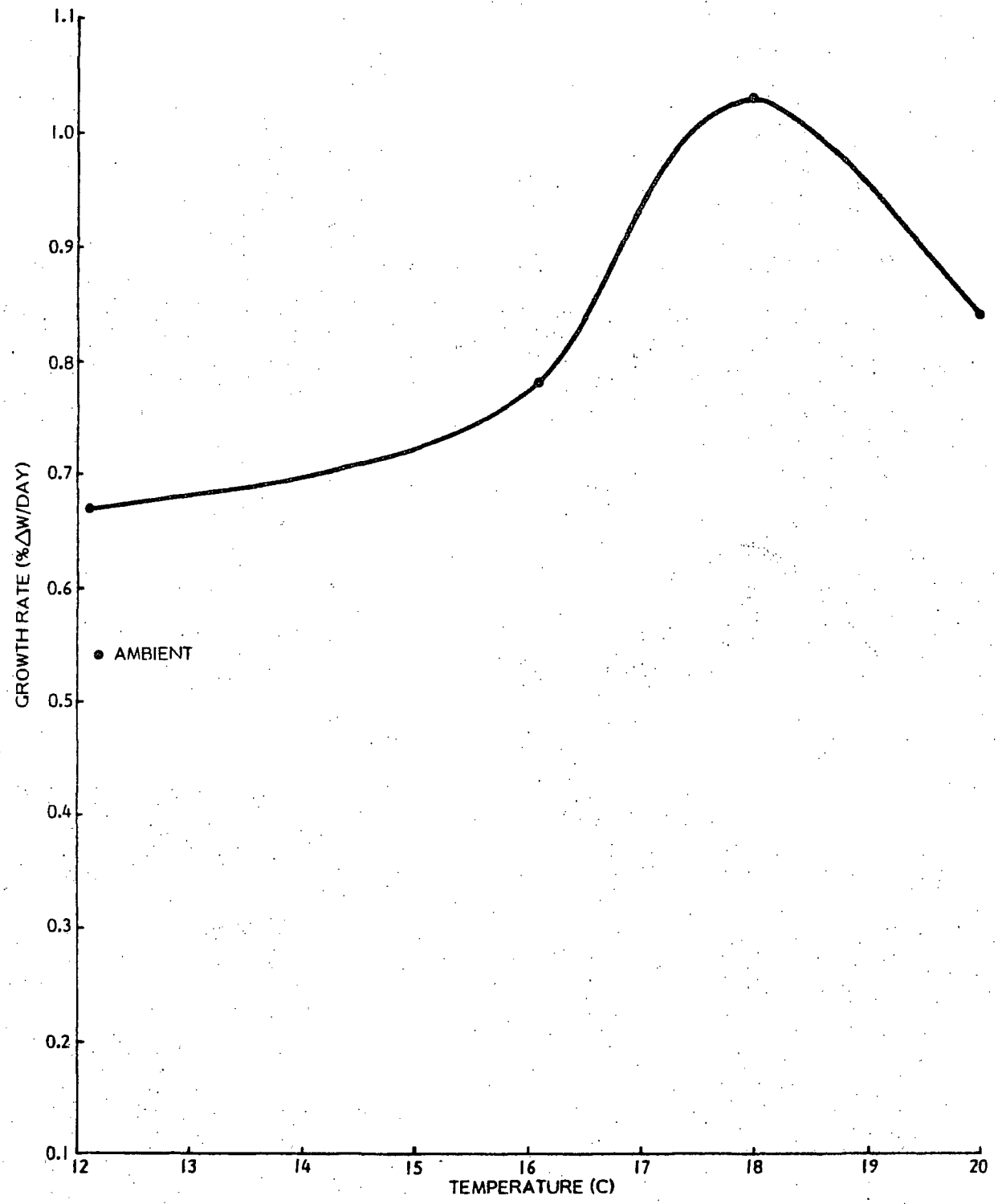


FIGURE 3.10.1-2

BLACK SURFPERCH OPTIMUM TEMPERATURE FOR GROWTH:
DAILY GROWTH RATE VERSUS TEMPERATURE

At the end of 59 days of exposure to the test temperatures the highest growth rate, 1.03 percentage weight increase per day, was exhibited by those fish maintained at 18.0 C. A substantial decrease in growth rate was recorded at only 2 C above and below this peak: 0.84 % ΔW/day at 20.0 C and 0.78 % ΔW/day at 16.1 C. Of all the constant temperature test populations, the lowest growth rate was exhibited by the animals at 12.2 C, 0.67 % ΔW/day.

Although the mean temperature in the ambient tank was 0.1 C greater than the 12.1 C tank (12.2 C) the growth rate for the animals at ambient was the lowest recorded for this experiment, 0.54 % W/day. The explanation for this lies in the daily temperatures experienced in this tank. On 35 of the 59 days of the experiment, daily mean temperatures were below 12 C. The lowest hourly temperature reading was 9.9 C and the mean temperature during the first month of growth was 11.5 C. Only 24 days had mean temperatures above 12 C. The highest temperature recorded was 16.2 C. From these data it would appear that under constant temperature conditions the optimum temperature for growth of juvenile black surfperch is close to or at 18 C when food is not limiting.

3.10.1.3 DISCUSSION

Under laboratory conditions, the optimum temperature for growth of black surfperch was estimated to be at or near 18 C (64 F). Substantially lower growth rates were recorded at 20.0 C and 16.2 C (68.0 and 61.2 F). There was some indication that at relatively low temperatures (12 C = 57.8 F) fluctuating temperature conditions result in lower growth rates than constant temperature conditions even when the overall mean temperature is the same.

These results indicate that elevated temperatures, up to approximately 18 C (64 F) could increase growth rates of resident black surfperch in Diablo Cove and that temperatures up to 20 C (68 F) would result in growth rates approximately equivalent to warm season (16 C = 61 F) growth rates in the Diablo Canyon area.

3.10.2 TEGULA BRUNNEA: GROWTH

A detailed review of the general biology, ecology, taxonomy and life history of the brown turban snail Tegula brunnea was presented by Morris et al. (1980). To orient the reader, a brief summary of the pertinent aspects of the biology of T. brunnea are presented here.

A common inhabitant of California kelp forests, T. brunnea ranges from Cape Arago, Oregon, to Santa Barbara, California. In Diablo Cove, T. brunnea is sparse in the low tidal zone but common at subtidal depths to 30 feet (Burge and Schultz 1973, North 1966). Lowry et al. (1974) reported that T. brunnea was most commonly found in the upper canopy portion of the giant kelp Macrocystis. They occasionally found it on the more benthic algae Dictyoneuropsis, Cystoseira and Gigartina. Hunt (1977) also observed T. brunnea in association with Macrocystis. He rarely observed it on the bottom.

Although no feeding studies have been performed on T. brunnea, Lowry et al. (1974) suggested that it is a herbivorous grazer. In the laboratory T. brunnea has been observed to consume Macrocystis, Nereocystis and diatoms growing on the walls of the aquarium (B. Tissot, personal observation). There are no known growth studies on this species.

The thermal tolerance of T. brunnea was investigated by PGandE (see SECTION 2.10.25). In that experiment, seawater temperatures exceeding 25.7 C for four days would cause approximately 50 percent of the exposed population to die. No mortality would be expected at and below 23.1 C for a four-day exposure.

The purpose of this experiment is to measure under laboratory conditions the growth rate of T. brunnea cultured in different combinations of temperature and algal diets. From this the optimal temperature and diet combination for growth can be obtained. In addition, the effects of the temperature and diet treatments on food consumption and mortality can be assessed. This information can provide an evaluation of the potential impact of changing ecological conditions which might be induced by the discharge of waste heat into Diablo Cove.

3.10.2.1 METHODS

This experiment was run concurrently on four snail species: the black abalone (Haliotis cracherodii), the red abalone (Haliotis rufescens), the black turban snail (Tegula funebralis) and the brown turban snail (Tegula brunnea). Methods were previously described in detail in SECTION 3.5.2.1 for the black abalone. A brief outline of the critical aspects of the materials and methods as they relate to T. brunnea is presented here.

Approximately 600 T. brunnea were collected on Dec. 12-13, 1978, between one and one-half miles south of Diablo Cove. The size range of individuals was from 5 to 37 mm in shell diameter. For a period of 30+ days the snails were held at ambient seawater temperatures and fed Macrocystis. During this time they were carefully examined for any injury due to handling.

At the end of the initial observation period the animals were individually tagged and placed in 12 inch pieces of 3 inch diameter PVC pipe covered at each end with a fine mesh plastic screen. An airstone was fitted in the middle of each pipe. These were placed in the appropriate 4x8 foot test tank. Flow rates in the test tanks were maintained at approximately 22.7 liters/min (6gpm). Test tank temperatures were slowly increased at the rate of 1 C/day until the appropriate test temperature was achieved. All tanks were monitored daily for mortalities. Each test tank was divided into three equal areas for the three diet treatments. Six test tanks were used -- one for each test temperature. Each diet compartment contained 30 T. brunnea. Each compartment also contained 30 T. funebralis, 30 Haliotis cracherodii and 16 H. rufescens. The two species of Tegula were contained in seperate habitats. Similar size distributions of T. brunnea were placed in each treatment. The total experimental population for the 18 treatment groups was 540 T. brunnea.

Test temperatures in each 4x8 tank were electronically controlled to within ±0.1 C of the specified test temperature throughout the course of the 90 day experiment. An unplanned power outage on day 6 caused larger temperature variations. The means, standard deviations, and ranges for the six temperature

treatments were: 12.1±0.7 (9.9-13.7; ambient control), 12.0±0.3 (11.7-13.6), 14.8±1.3 (13.3-19.8), 17.9±0.4 (13.3-18.4), 21.0±1.2 (15.1-21.8), and 23.8±0.9 (14.2-24.2). The 90-day mean temperature for each treatment will be used for identification in this report.

The three algal diets were composed of brown algae (Nereocystis luetkeana and Pterygophora californica), red algae (Iridaea flaccida and Botryoglossum farlowianum) and a mixed diet composed of roughly equal amounts of the four preceding algal species. The algal species were selected to represent the species of algae which commonly compose the algal drift found along the Diablo Cove shoreline. All three diets were fed twice a week to excess in each treatment group.

Twice during the experiment (day 17 and day 70) consumption was monitored over a three day period. This consisted of weighing the algae before and after the trial period. The difference was assumed to be the amount consumed. The wet caloric values of Paine and Vadas (1969) were used to estimate caloric intake. The dry caloric value of Cryptopleura violacea was substituted for Botryoglossum farlowianum. From the dry caloric value and percent water, the wet caloric value of Cryptopleura was calculated. Percent water was calculated from information made available by the California Department of Fish and Game at Diablo Canyon.

Maximum shell diameter and total weight were recorded at day 1, 49 and 91. In addition, subsamples of body component weights in each test population were collected at the beginning and end of the experiment.

3.10.2.2 RESULTS

Preliminary results indicate that changes in total body weight and body component weights are smaller among treatments in comparison to differences in maximum shell diameter. Therefore, in the final analysis which follows, only changes in shell diameter are used. Results for mortality and consumption are also presented.

Mortality

Mortality rates among T. brunnea were 6.5 percent overall (TABLE 3.10.2-1). A two-way analysis of variance (ANOVA) discloses significant differences in mortality among temperatures ($p < 0.01$) but not diets ($p=0.63$). Results of a Student-Newman-Keuls (SNK) multiple range test (TABLE 3.10.2-1) separates the temperatures into three subsets: 23.8, 21.0 and the range 12.0-17.9 C. The highest mortality occurred at 23.8 C, the second highest at 21.0 C. Mortality was low within the range 12.0-17.9 C. Mortalities occurred throughout the duration of the experiment.

Consumption

Results of a two-way ANOVA (TABLE 3.10.2-2) reveal significant differences in consumption among diets but not temperatures. An SNK multiple range test seperates two homogeneous diet subsets (TABLE 3.10.2-2 and FIGURE 3.10.2-1). The brown and mixed algal diets were consumed more readily than the red algal diet. As food preferences of T. brunnea have not been investigated, it is of interest to compare consumption among the algae in the mixed diet. A two way ANOVA reveals significant differences in consumption among algae, but not temperatures in the mixed diet (TABLE 3.10.2-3). An SNK multiple range test shows that Nereocystis is consumed much more readily than Botryoglossum, Iridaea, or Pterygophora (TABLE 3.10.2-3).

When the consumption data were transformed to caloric values, differences among diets but not temperatures were significant (TABLE 3.10.2-4). An SNK multiple range test reveals two homogeneous treatment subsets (TABLE 3.10.2-4 and FIGURE 3.10.2-2). Results are identical to that for consumption. Thus, caloric consumption is very similar to overall consumption in T. brunnea.

Growth

As the T. brunnea were measured after two approximately equal periods of growth, it is of interest to compare the results of these periods seperately. A

TABLE 3.10.2-1

MORTALITY AMONG TREATMENTS:
TEGULA BRUNNEA GROWTH

Mean Temperature (C)	Experimental Group		
	Brown	Red	Mixed
12.0	1	0	0
12.1*	0	0	0
14.8	0	0	1
17.9	0	0	0
21.0	0	4	0
23.8	8	9	12

*Ambient Control

Student-Newman-Keuls Multiple Range Test*

Treatment (C)					
23.8	21.0	14.8	12.0	17.9	12.1

* Treatments are ordered according to the magnitude of their mean value. Treatments connected by underline are not significantly different.

TABLE 3.10.2-2

TESTS OF SIGNIFICANCE OF
CONSUMPTION DATA: TEGULA BRUNNEA GROWTH

Two-Way Analysis of Variance

Source	Degrees of Freedom	Sum of Squares	Mean Squares	F Value
Diet	2	1,233.4	616.7	17.3*
Temperature	5	513.3	102.7	2.9
Error	10	356.8	35.7	

* Significant F-value at $\alpha = 0.05$

Student-Newman-Keuls Multiple Range Test*

Treatment		
Mix	Brown	Red

* Treatments are ordered according to the magnitude of their mean value. Treatments connected by underline are not significantly different.

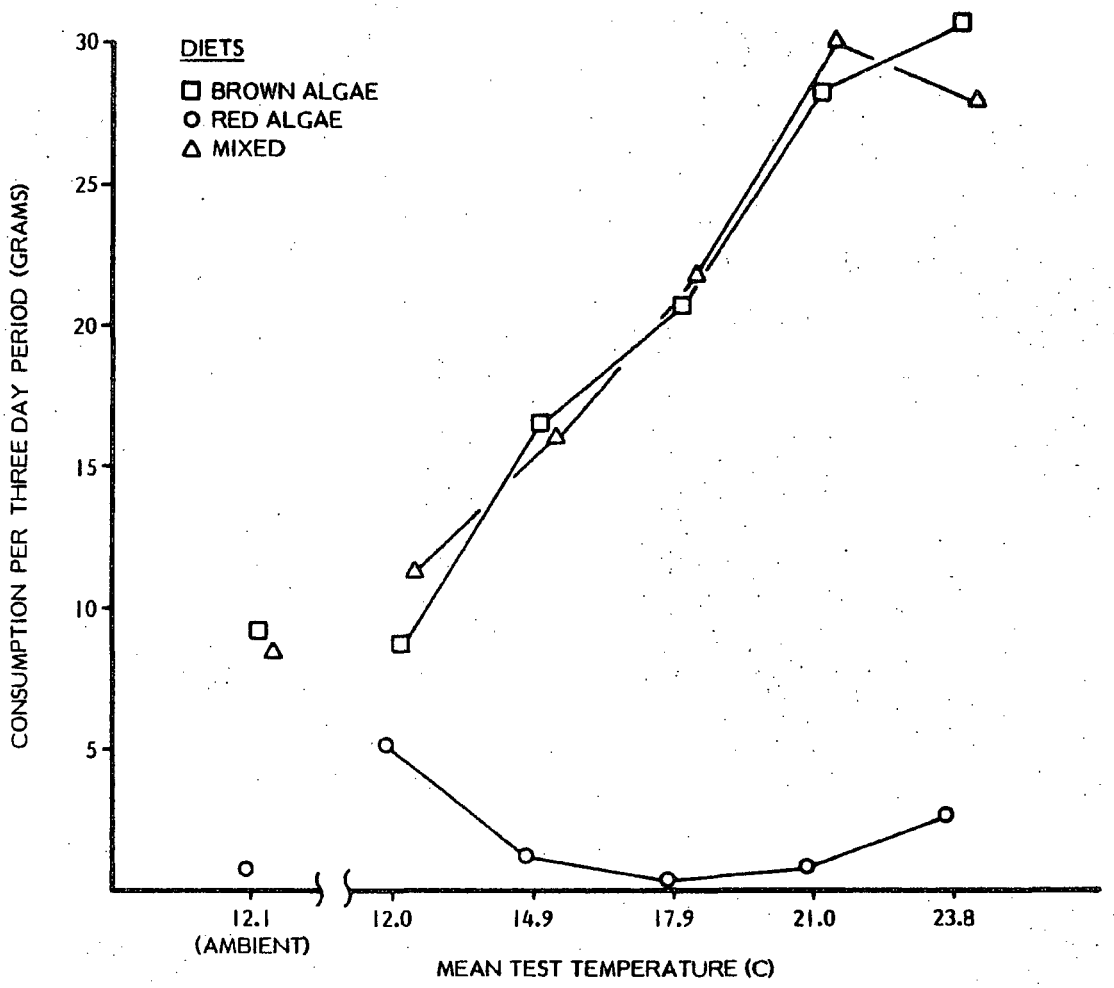


FIGURE 3.10.2-1

CONSUMPTION VERSUS TEMPERATURE:
TEGULA BRUNNEA GROWTH

TABLE 3.10.2-3

TESTS OF SIGNIFICANCE OF CONSUMPTION AMONG ALGAE IN MIXED DIET: TEGULA BRUNNEA GROWTH

Two-Way Analysis of Variance

Source	Degrees of Freedom	Sum of Squares	Mean Squares	F Value
Algae	3	6,046.4	2,015.5	24.85*
Temperature	5	391.3	78.3	0.96
Error	15	1,216.4	81.1	

* Significant F-value at $\alpha = 0.05$

Student-Newman-Keuls Multiple Range Test*

Treatment

Nereocystis	Botryoglossum	Iridaea	Pterygophora

* Treatments are ordered according to the magnitude of their mean value. Treatments connected by underline are not significantly different.

TABLE 3.10.2-4

TESTS OF SIGNIFICANCE OF CALORIC CONSUMPTION DATA: TEGULA BRUNNEA GROWTH

Two-Way Analysis of Variance

Source	Degrees of Freedom	Sum of Squares	Mean Squares	F Value
Diet	2	155.6	77.8	9.58*
Temperature	5	72.1	14.4	1.77
Error	10	81.2	8.1	

* Significant F-value at $\alpha = 0.05$

Student-Newman-Keuls Multiple Range Test*

Treatment

Mix Brown Red

* Treatments are ordered according to the magnitude of their mean value. Treatments connected by underline are not significantly different.

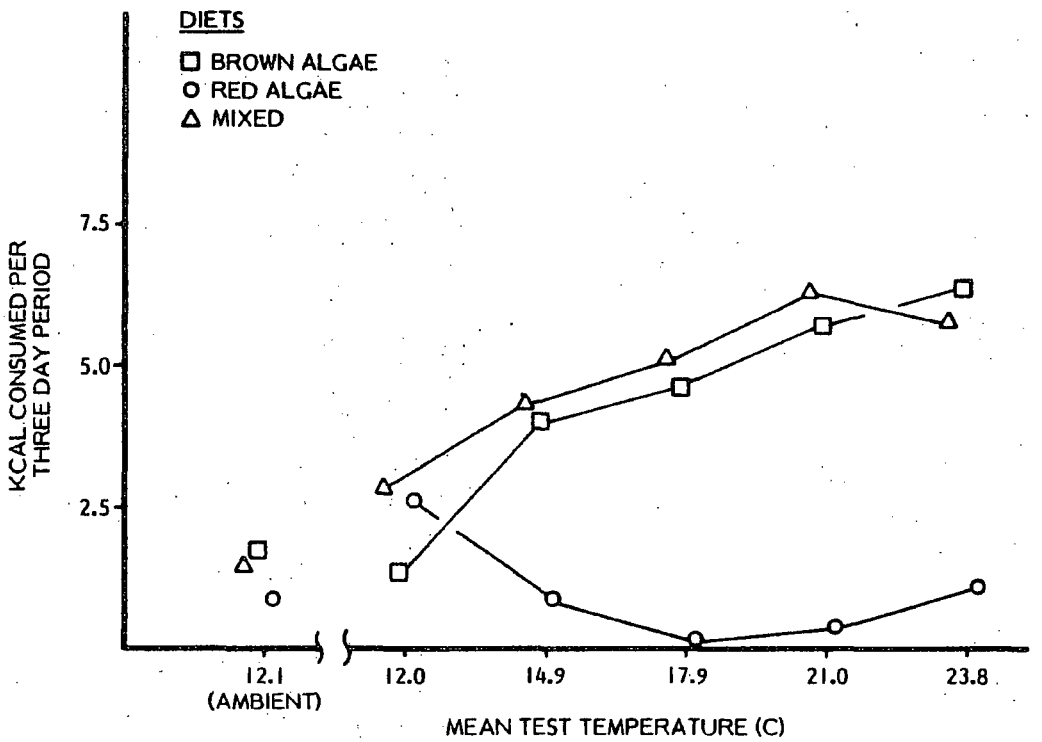


FIGURE 3.10.2-2

CALORIC CONSUMPTION VERSUS TEMPERATURE:
TEGULA BRUNNEA GROWTH

preliminary analysis of the homogeneity of group variances (Bartlett-Box, Cochrans and F-Max tests) indicated that group dispersions were unequal. To satisfy this important assumption of the ANOVA the data was transformed to a logarithmic (base 10) scale.

Results for the two growth periods are the same (TABLES 3.10.2-5 and 3.10.2-6). For both growth periods, significant differences exist among diets, temperatures, and the diet-temperature interaction. An SNK multiple range test on the 90 day means separates the treatments into four homogeneous subsets (TABLE 3.10.2-7). These subsets form groups contrasting differences in diet and temperature. Shell growth is greatest on the brown and mixed algal diets at 12.0-21.0 C temperatures. This is contrasted with growth on the brown and mixed algal diets at 23.8, and on the red algal diet at all temperatures.

The origin of the interaction can be seen in FIGURE 3.10.2-3. It appears that the response of shell growth in T. brunnea to temperature is not the same on all diets. The brown and mixed algal diets are very similar: growth increases with temperature, is maximal between 14.8 and 21.0 C, and declines at 23.8 C. On the red algal diet growth is minimal at 17.9 and 12.1 C (ambient); increasing as the temperature increases or decreases from these points.

To test whether different size distributions in the treatments were influencing the results, an analysis of covariance was performed using initial weight as the covariate. The results are in TABLES 3.10.2-8 and 3.10.2-9 for the two growth periods. For both analyses the covariates are significant, but they do not change the significance of the results. From this it can be inferred that changes in shell diameter are directly related to the initial shell diameter. Due to similar size distributions in the treatments, however, adjustment of shell growth relative to initial size does not change the overall results.

3.10.2.3. DISCUSSION

Mortality rates among T. brunnea in this experiment are consistent with the findings of PGandE (1979b). The highest mortality (32 percent) occurred at the

TABLE 3.10.2-5

TESTS OF SIGNIFICANCE OF GROWTH FOR DAYS 1-49: *TEGULA BRUNNEA* GROWTH

Two-Way Analysis of Variance

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Significance of F
Main Effects					
Temperature	5	6.334	1.267	12.131	0.000
Diet	2	9.703	4.852	46.459	0.000
2-Way Interactions					
Temperature and Diet	10	2.457	0.246	2.353	0.010
Residual	420	43.859	0.104		
Total	437	62.421	0.143		

* Significant F-value at $\alpha = 0.05$

TABLE 3.10.2-6

TESTS OF SIGNIFICANCE OF GROWTH FOR DAYS 49-91: *TEGULA BRUNNEA* GROWTH

Two-Way Analysis of Variance

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Significance of F
Main Effects					
Temperature	5	5.763	1.153	12.667	0.000
Diet	2	25.386	12.693	139.490	0.000
2-Way Interactions					
Temperature and Diet	10	1.947	0.195	2.139	0.021
Residual	420	38.218	0.091		
Total	437	71.254	0.163		

* Significant F-value at $\alpha = 0.05$

TABLE 3.10.2-7

TEST OF SIGNIFICANCE OF
90-DAY GROWTH: TEGULA BRUNNEA GROWTH

Student-Newman-Keuls Multiple Range Test*

Treatment Groups**

16	4	5	17	15	3	14	2	1	13	6	11	9	12	8	18	10	7

* Treatments are ordered according to the magnitude of their mean value. Treatments connected by underline are not significantly different.

** Treatment Group Codes

	Temp (C)					
	12.1	12.0	14.8	17.9	21.0	23.8
Brown Algal Diet	1	2	3	4	5	6
Red Algal Diet	7	8	9	10	11	12
Mixed Algal Diet	13	14	15	16	17	18

Student-Newman-Keuls Multiple Range Test*

Treatment Groups**

4	16	5	15	17	14	3	2	1	13	18	6	11	8	12	9	10	7

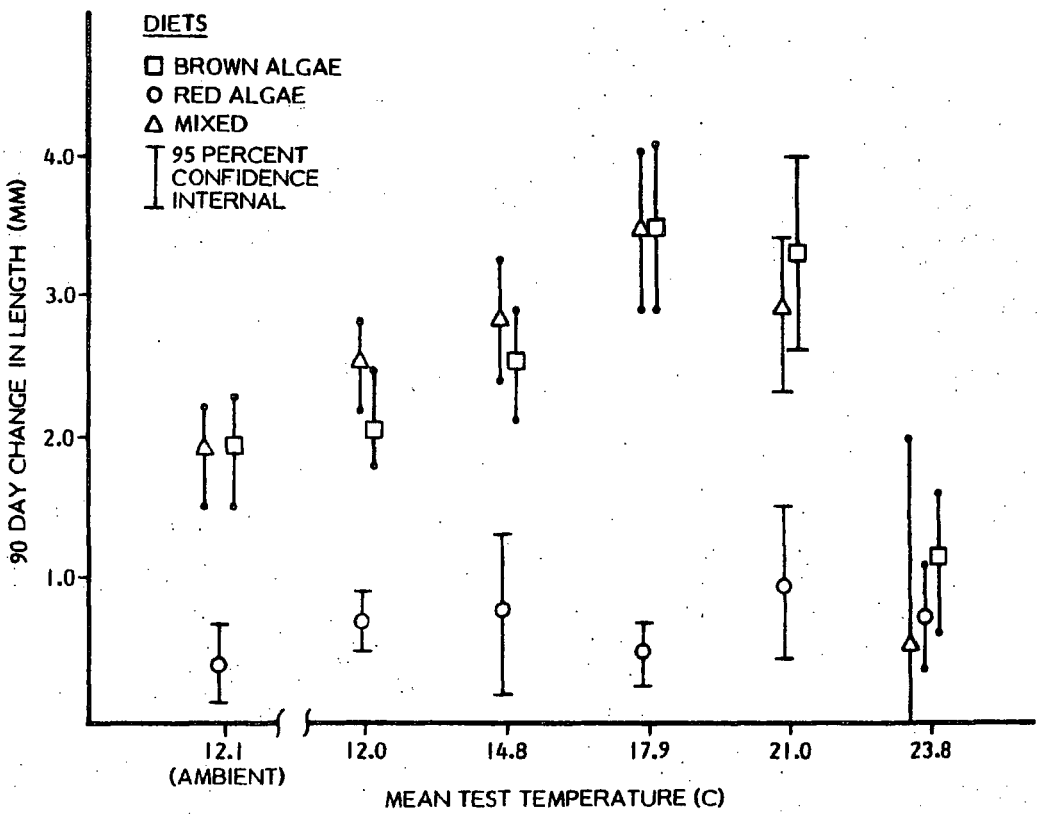


FIGURE 3.10.2-3

90-DAY GROWTH VERSUS TEMPERATURE:
TEGULA BRUNNEA GROWTH

TABLE 3.10.2-8

TWO-WAY ANALYSIS OF VARIANCE OF GROWTH ADJUSTED BY INITIAL SHELL DIAMETER FOR DAYS 1-49: *TEGULA BRUNNEA* GROWTH

Two-Way Analysis of Variance

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Significance of F
Covariates					
LNI	1	2.935	2.935	30.512	0.000*
Main Effects					
Temperature	5	6.962	1.392	14.475	0.000*
Diet	2	9.661	4.831	50.219	0.000*
2-Way Interactions					
Temperature and Diet	10	2.490	0.249	2.589	0.005*
Residual	419	40.305	0.096		
Total	437	62.421	0.143		

* Significant F-value at $\alpha = 0.05$

TABLE 3.10.2-9

TWO-WAY ANALYSIS OF VARIANCE OF GROWTH ADJUSTED BY INITIAL SHELL DIAMETER FOR DAYS 49-91: TEGULA BRUNNEA GROWTH

Two-Way Analysis of Variance

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Significance of F
Covariates					
LN2	1	3.001	3.001	38.069	0.000*
Main Effects					
Temperature	5	6.718	1.344	17.044	0.000*
Diet	2	26.725	13.362	169.517	0.000*
2-Way Interactions					
Temperature and Diet	10	2.016	0.202	2.558	0.005*
Residual	419	33.028	0.079		
Total	437	71.254	0.163		

* Significant F-value at $\alpha = 0.05$

highest temperature: 23.8 C. This approaches the ET50 value (i.e. the temperature at which 50 percent of the population survive) of 25.7 C determined by PGandE (1979b).

As temperatures increased, consumption also increased. This suggests that T. brunnea is trying to maintain a constant energy balance as their metabolic rate increases. Caloric consumption also follows this trend. The agreement between consumption and caloric consumption suggests that T. brunnea does not regulate its caloric intake, but consumes the alga that is the most preferred.

The similarities between consumption of the brown and mixed algal diets is due to the preferences of T. brunnea. Individuals feeding on the mixed diet showed a distinct preference for Nereocystis. In fact, Nereocystis represented 95 percent of their total intake on this diet. Individuals feeding on the brown algal diet also consumed primarily Nereocystis. Thus, these diets were essentially identical, resulting in similar consumption, caloric consumption and growth.

In this experiment, optimum shell growth in T. brunnea occurred while consuming the brown and mixed algal diets at 17.9 C (64.2 F) (TABLE 3.10.2-7 and FIGURE 3.10.2-3). This is slightly above the average annual temperature found in Diablo Cove. Shell growth at 17.9 C (64.2 F) for 90 days averaged 3.5 mm (14 mm/year) on the brown and mixed algal diets; with some individuals growing as much as 6 mm. Growth rates of T. funebralis, a closely related species, have been reported at 2.2 mm/year (Peppard 1964). Thus, T. brunnea grows much faster that its intertidal congener T. funebralis.

That both diet and temperature had significant effects on the growth of T. brunnea is of interest. The major effect of diet was due primarily to the preference of T. brunnea for Nereocystis, and their elevated consumption on this diet. From the data it appears that the conversion efficiency (growth per unit consumption) is approximately the same on all diets. Studies on T. funebralis indicate that it can digest a large variety of algal nutrients (Galli and Giese 1959). If T. brunnea has a similar adaptation, it is likely that it could exist on any available algae.

3.10.3 TEGULA FUNEBRALIS GROWTH

A review of the general biology, ecology, taxonomy and life history of the black turtan snail, Tegula funebralis, was presented by Morris et al. (1980). This species is abundant in the intertidal zone at Diablo Canyon. A detailed review of the literature on T. funebralis was presented in PGand E (1979b).

T. funebralis is a very slow growing species. Individuals are commonly 20-30 years old (Darby 1964). Shell growth appears to cease in larger individuals from November to February, thus producing an annual growth ring in the shell (Paine 1969). In Sunset Bay, Oregon, Darby (1964) found shell growth rates of 0.4 mm/year from individuals tagged and recovered in the field. In a laboratory study, Peppard (1964) calculated growth rates of 2.2 mm/year for a Pacific Grove population. Frank (1975) found widely variable growth rates between populations of T. funebralis. He interpretated this variation as an ecological cline along the Pacific coast. Southern populations were found to be composed of smaller individuals that grow at a faster rate, and hence live a shorter life than populations to the north. Water temperature appears to be the major factor controlling this cline, and hence the growth of T. funebralis (Frank 1975).

The thermal tolerance of T. funebralis was investigated by PGandE (1979b). It was predicted that seawater temperatures exceeding 28.8 C for four days would cause approximately 60 percent of the exposed population to die. No mortality would be expected at and below 28.1 C for a four day exposure.

The purpose of this experiment was to measure under laboratory conditions the growth rate of T. funebralis cultured in different combinations of temperature and algal diets. From this the optimal temperature and diet combination for growth can be obtained. In addition, the effects of the temperature and diet treatments on food consumption and mortality can be assessed. This information can provide an evaluation of the potential impact of changing ecological conditions which might be induced by the discharge of waste heat into Diablo Cove.

3.10.3.1 METHODS

This experiment was run concurrently on four snail species: the black abalone, Haliotis cracherodii, the red abalone, Haliotis rufescens; the brown turban snail, Tegula brunnea and the black turban snail, Tegula funebralis. Methods were previously described in detail in SECTION 3.5.2.1 for the black abalone. A brief outline of the critical aspects of the materials and methods as they relate to T. funebralis is presented here.

Approximately 600 T. funebralis were collected on December 12-13, 1978, between one and one-half miles south of Diablo Cove. The size range of individuals was from 5 to 30 mm in shell diameter. For a period of 30+ days the snails were held at ambient seawater temperatures and fed Macrocystis. During this time they were carefully examined for any injury due to handling.

At the end of the initial observation period the animals were individually tagged and placed in 12 inch pieces of 3 inch diameter PVC pipe covered at each end with a fine mesh plastic screen. An airstone was fitted in the middle of each pipe. These were placed in the appropriate 4x8 foot test tank. Flow rates in the test tanks were maintained at approximately 22.7 liters/min (6gpm). Test tank temperatures were slowly increased at the rate of 1 C/day until the appropriate test temperature was achieved. All tanks were monitored daily for mortalities. Each test tank was divided into three equal areas for the three diet treatments. Six test tanks were used -- one for each test temperature. Each diet compartment contained 30 T. funebralis. Each compartment also contained 30 T. brunnea, 30 Haliotis cracherodii and 16 Haliotis rufescens. The two species of Tegula were contained in seperate habitats. Similar size distributions of T. funebralis were placed in each treatment. The total experimental population for the 18 treatment groups was 540 T. funebralis.

Test temperatures in each 4x8 tank were electronically controlled to within ±0.1 C of the specified test temperature throughout the course of the 90 day experiment. An unplanned power outage on day 6 caused larger temperature variations. The means, standard deviations, and ranges for the six temperature

treatments were: 12.1±0.7 (9.9-13.7; ambient control), 12.0±0.3 (11.7-13.6), 14.8±1.3 (13.3-19.8), 17.9±0.4 (13.3-18.4), 21.0±1.2 (15.1-21.8), and 23.8±0.9 (14.2-24.2). The 90 day mean temperature for each treatment will be used for identification in this report.

The three algal diets were composed of brown algae (Nereocystis luetkeana and Pterygophora californica), red algae (Iridaea flaccida and Botryoglossum farlowianum) and a mixed diet composed of roughly equal amounts of the four preceding algal species. The algal species were selected to represent the species of algae which commonly compose the algal drift found along the Diablo Cove shoreline. All three diets were fed twice a week to excess in each treatment group.

Twice during the experiment (day 17 and day 70) consumption was monitored over a three day period. This consisted of weighing the algae before and after the trial period. The difference was assumed to be the amount consumed. The wet caloric values of Paine and Vadas (1969) were used to estimate caloric intake. The dry caloric value of Cryptopleura violacea was substituted for that of Botryoglossum farlowianum. From the dry caloric value and percent water, the wet caloric value of Cryptopleura was calculated. Percentage water was calculated from information made available by the California Department of Fish and Game at Diablo Canyon.

Maximum shell diameter and total weight were recorded at day 1, 49 and 91. In addition, subsamples of body component weights in each test population were collected at the beginning and end of the experiment.

3.10.3.2 RESULTS

Preliminary results indicate that changes in the maximum shell diameter and body component weights are smaller among treatments in comparison to differences in total body weight. Therefore, in the final analysis which follows, only changes in body weight are used. Results for mortality and consumption are also presented.

Mortality

Mortality rates among T. funebralis were 2.6 percent overall (TABLE 3.10.3-1). A two-way analysis of variance (ANOVA) discloses significant differences in mortality among temperatures ($p<0.01$) but not diets ($p=0.39$). Results of a Student-Newman-Keuls (SNK) multiple range test (TABLE 3.10.3-1) separates the temperatures into 3 subsets: 21.0, 23.8 and the range 12.0-17.9 C. The highest mortality occurred at 21.0 C, the second highest at 23.8 C. No mortality occurred within the range 12.0-17.9 C. Except for two individuals, all mortalities occurred in the first 30 days of the experiment.

Consumption

Results of a two-way ANOVA (TABLE 3.10.3-2) reveal significant differences in consumption among diets and temperatures (both $p<0.05$). An SNK multiple range test seperates 3 homogeneous subsets (TABLE 3.10.3-2 and FIGURE 3.10.3-1). The primary pattern of grouping pertains to diet -- the brown and mixed algal diets were consumed the most. A secondary pattern contrasts elevated consumption at 17.9-23.8 C with lower temperatures.

When the consumption data was transformed to caloric values, differences among diets and temperatures were significant (TABLE 3.10.3-3). An SNK multiple range test reveals three homogeneous treatment subsets (TABLE 3.10.3-3 and FIGURE 3.10.3-2). Groupings pertain primarily to diet and secondarily to temperature. Caloric consumption is very similar to overall consumption in T. funebralis.

Growth

As the T. funebralis were measured after two approximately equal periods of growth, it is of interest to compare the results of these periods seperately. A preliminary analysis of the homogeneity of group variances (Bartlett-Box, Cochrans and F-Max tests) indicated that group dispersions were unequal. To

TABLE 3.10.3-1

MORTALITY AMONG TREATMENTS:
TEGULA FUNEBRALIS GROWTH

Mean Temperature (C)	Diet		
	Brown	Red	Mixed
12.0	0	0	0
12.1*	0	0	0
14.8	0	0	0
17.9	0	0	0
21.0	2	3	5
23.8	0	3	1

*Ambient Control

Student-Newman-Keuls Multiple Range Test*

Treatment (C)					
21.0	23.8	17.9	14.8	12.1	12.0

* Treatments are ordered according to the magnitude of their mean value. Treatments connected by underline are not significantly different.

TABLE 3.10.3-2

TESTS OF SIGNIFICANCE OF CONSUMPTION DATA: TEGULA FUNEBRALIS GROWTH

Two-Way Analysis of Variance

Source	Degrees of Freedom	Sum of Squares	Mean Squares	F Value
Diet	2	548.4	274.2	9.06*
Temperature	5	719.5	143.9	4.75*
Error	10	302.8	30.3	

* Significant F-value at $\alpha = 0.05$

Student-Newman-Keuls Multiple Range Test*

Treatment Groups**

6	18	16	5	13	17	4	15	3	12	1	7	2	14	11	9	8	10

* Treatments are ordered according to the magnitude of their mean value. Treatments connected by underline are not significantly different.

** Treatment Group Codes

	Temp (C)					
	12.1	12.0	14.8	17.9	21.0	23.8
Brown Algal Diet	1	2	3	4	5	6
Red Algal Diet	7	8	9	10	11	12
Mixed Algal Diet	13	14	15	16	17	18

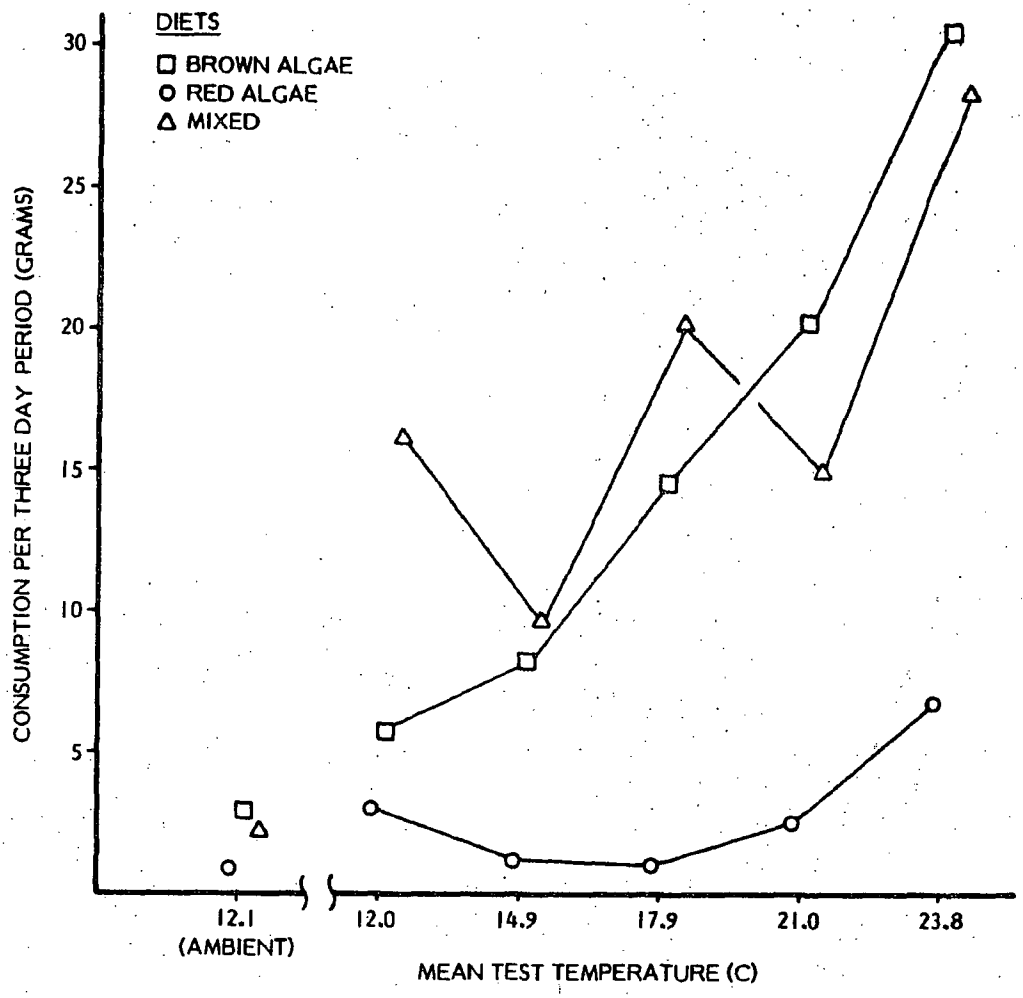


FIGURE 3.10.3-1

CONSUMPTION VERSUS TEMPERATURE:
TEGULA FUNEBRALIS GROWTH

TABLE 3.10.3-3

TESTS OF SIGNIFICANCE OF CALORIC CONSUMPTION DATA: TEGULA FUNEBRALIS GROWTH

Two-Way Analysis of Variance

Source	Degrees of Freedom	Sum of Squares	Mean Squares	F Value
Diet	2	42.32	21.2	4.37*
Temperature	5	147.30	29.5	6.08*
Error	10	48.4	4.8	

* Significant F-value at $\alpha = 0.05$

Student-Newman-Keuls Multiple Range Test*

Treatment Groups**

6	18	13	16	4	5	12	17	15	7	11	3	1	9	8	10	2	14

* Treatments are ordered according to the magnitude of their mean value. Treatments connected by underline are not significantly different.

** Treatment Group Codes

	Temp (C)					
	12.1	12.0	14.8	17.9	21.0	23.8
Brown Algal Diet	1	2	3	4	5	6
Red Algal Diet	7	8	9	10	11	12
Mixed Algal Diet	13	14	15	16	17	18

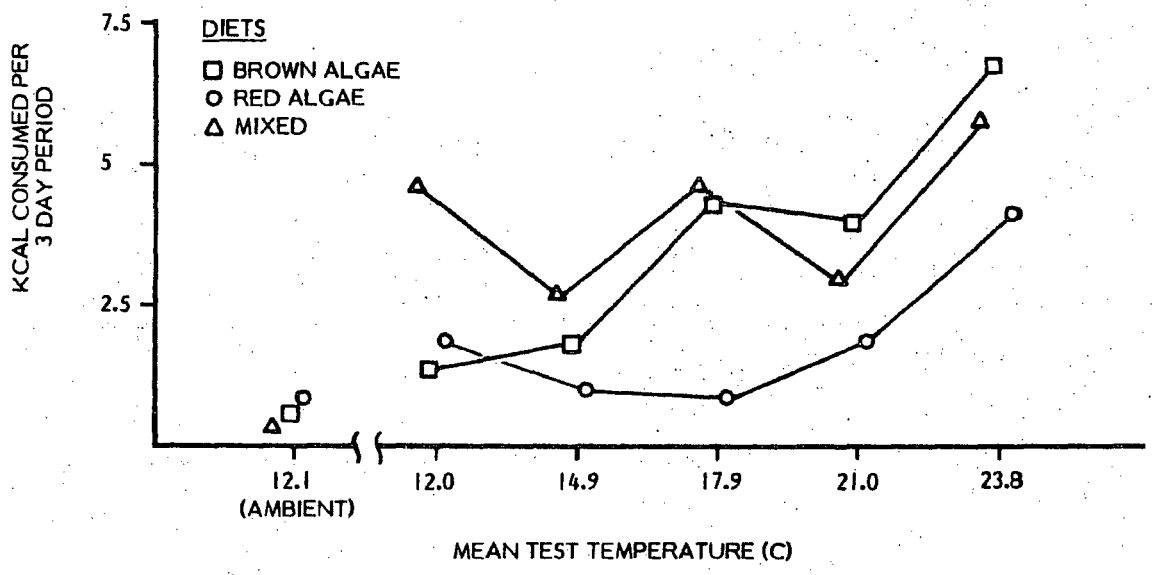


FIGURE 3.10.3-2

CALORIC CONSUMPTION VERSUS TEMPERATURE:
TEGULA FUNEBRALIS GROWTH

satisfy this important assumption of the ANOVA the data was transformed to a logarithmic (base 10) scale.

Results for the two growth periods are the same (TABLES 3.10.3-4 and 3.10.3-5). For both growth periods, significant differences exist among diets and temperatures (both $p < 0.01$). An SNK multiple range test on the 90 day means separates the treatments into eight homogeneous subsets (TABLE 3.10.3-6). These subsets form two major groups contrasting differences in diet and temperature. Body growth is greatest on the brown and mixed algal diets at 14.8-23.8 C temperatures. This contrasts with growth on the brown and mixed algal diets at 12.0 and 12.1 C (ambient), and on the red algal diet at all temperatures.

To test whether different size distributions in the treatments were influencing the results, an analysis of covariance was performed using initial weight as the covariate. The results are in TABLES 3.10.3-7 and 3.10.3-8 for the two growth periods. For both analyses the covariates are significant, but they do not change the significance of the main factors . However, in the first growth period the interaction between diet and temperature becomes significant. The origin of this interaction can be seen in FIGURE 3.10.3-3. It appears that the response of growth in *T. funebralis* to temperature is not the same on all diets. The brown and mixed algal diets are very similar: growth increases with temperature and is maximal between 14.8 and 23.8 C. There appears to be no increase in growth with temperature on the red algal diet.

From the significance of the covariates it can be inferred that changes in body weight are directly related to the initial body weight. Due to slight differences in the size distributions in the treatments, adjustment of body weight growth relative to initial size caused changes in the significance of the results. Variation was partitioned from the error term to the covariate, thus bringing the diet-temperature interaction term to a significant level.

TABLE 3.10.3-4

TESTS OF SIGNIFICANCE OF GROWTH FOR DAYS 1-49: *TEGULA FUNEBRALIS* GROWTH

Two-Way Analysis of Variance

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Significance of F
Main Effects					
Temperature	5	8.163	1.633	16.849	0.000*
Diet	2	3.393	1.696	17.506	0.000*
2-Way Interactions					
Temperature and Diet	10	1.755	0.176	1.812	0.056
Residual	477	46.220	0.097		
Total	494	59.437	0.120		

* Significant F-value at $\alpha = 0.05$

TABLE 3.10.3-5

TESTS OF SIGNIFICANCE OF GROWTH FOR DAYS 49-91: *TEGULA FUNEBRALIS* GROWTH

Two-Way Analysis of Variance

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Significance of F
Main Effects					
Temperature	5	7.151	1.430	15.306	0.000*
Diet	2	3.934	1.967	21.051	0.000*
2-Way Interactions					
Temperature and Diet	10	1.370	0.137	1.466	0.149
Residual	477	44.568	0.093		
Total	494	57.050	0.115		

* Significant F-value at $\alpha = 0.05$

TABLE 3.10.3-6

TEST OF SIGNIFICANCE OF 90-DAY GROWTH: TEGULA FUNEBRALIS GROWTH

Student-Newman-Keuls Multiple Range Test*

Treatment Groups**

5	17	16	18	6	4	15	3	12	13	14	1	2	10	9	11	7	8
———	———	———	———	———	———												
	———	———	———	———	———	———											
			———	———	———	———	———										
						———	———	———									
							———	———	———	———	———	———	———	———			
									———	———	———	———	———	———	———		
										———	———	———	———	———	———	———	
															———	———	

* Treatments are ordered according to the magnitude of their mean value. Treatments connected by underline are not significantly different.

** Treatment Group Codes

	Temp (C)					
	12.1	12.0	14.8	17.9	21.0	23.8
Brown Algal Diet	1	2	3	4	5	6
Red Algal Diet	7	8	9	10	11	12
Mixed Algal Diet	13	14	15	16	17	18

TABLE 3.10.3-7

TWO-WAY ANALYSIS OF VARIANCE OF GROWTH ADJUSTED BY INITIAL WEIGHT FOR DAYS 1-49: TEGULA FUNEBRALIS GROWTH

Two-Way Analysis of Variance

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Significance of F
Covariates					
WT1	1	14.981	14.981	211.301	0.000*
Main Effects					
Temperature	5	6.346	1.269	17.901	0.000*
Diet	2	2.947	1.474	20.785	0.000*
2-Way Interactions					
Temperature and Diet	10	1.472	0.147	2.077	0.025*
Residual	476	33.747	0.071		
Total	494	59.437	0.120		

* Significant F-value at $\alpha = 0.05$

TABLE 3.10.3-8

TWO-WAY ANALYSIS OF VARIANCE OF GROWTH ADJUSTED BY INITIAL WEIGHT FOR DAYS 49-91: *TEGULA FUNEBRALIS* GROWTH

Two-Way Analysis of Variance

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Significance of F
Covariates					
WT2	1	5.400	5.400	62.506	0.000*
Main Effects					
Temperature	5	5.891	1.178	13.638	0.000*
Diet	2	3.403	1.701	19.694	0.000*
2-Way Interactions					
Temperature and Diet	10	1.243	0.124	1.439	0.160
Residual	476	41.125	0.086		
Total	494	57.050	0.115		

* Significant F-value at $\alpha = 0.05$

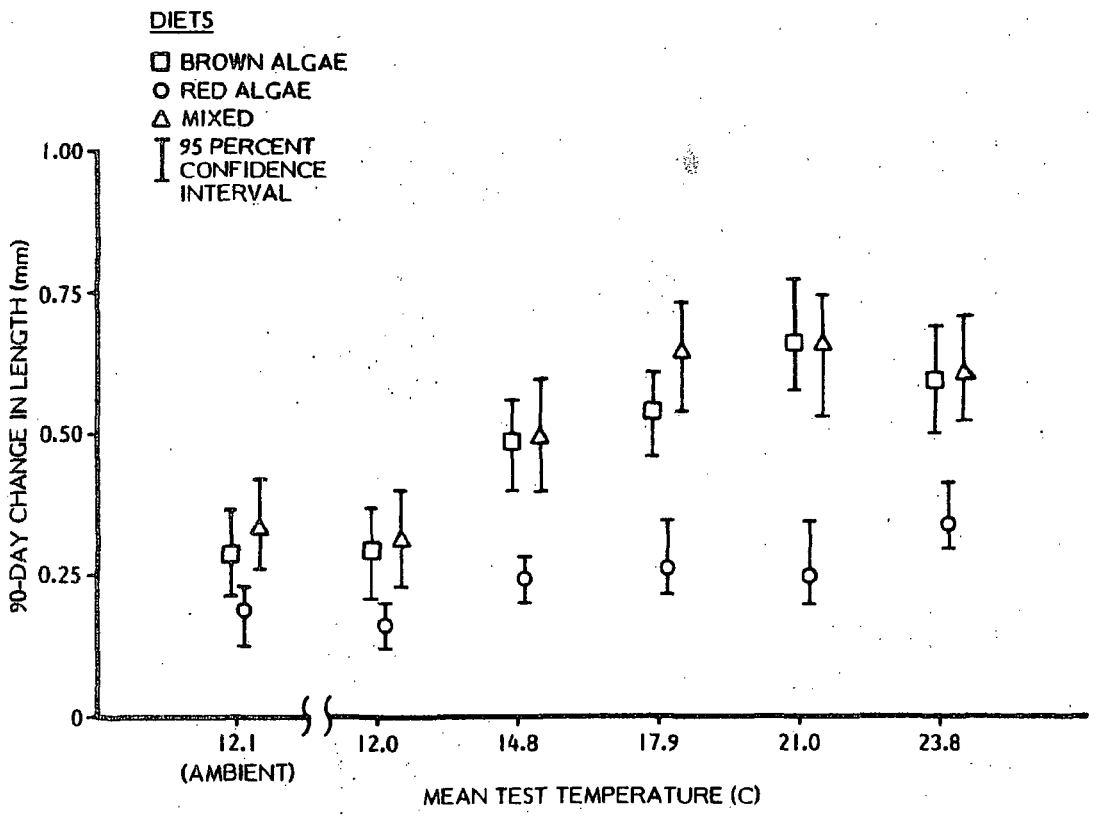


FIGURE 3.10.3-3

90-DAY GROWTH VERSUS TEMPERATURE:
TEGULA FUNEBRALIS GROWTH

3.10.3.3 DISCUSSION

PGandE (1979b) reported that T. funebralis is tolerant of temperatures up to 28.1 C (82.6F). In this growth experiment, mortality rates were low overall but there were significant differences among 12.0-23.8 C (53.6-74.8 F) temperatures. That mortality at 21.0 C (69.8 F) was greater than that at 23.8 C (74.8 F) suggests that temperature may not be the controlling factor.

The similarity between the brown and mixed algal diets in terms of consumption is due to the preferences of T. funebralis. It has been shown that T. funebralis prefers brown over red algae (Best 1964). The group that was fed the mixed diet consistently consumed the brown algal portion first, and this constituted the majority of their dietary intake. Their apparent dislike for red algae accounts for its low degree of consumption.

As temperatures increased, consumption also increased. This suggests that T. funebralis is trying to maintain a constant energy balance as their metabolic rate increases. Caloric consumption also follows this trend. The agreement between consumption and caloric consumption suggests that T. funebralis does not regulate its caloric intake, but consumes the alga that is the most preferred.

In this experiment, optimum body growth in T. funebralis occurred while consuming the brown and mixed algal diets at 14.8-23.8 C (58.6-74.8 F) (FIGURE 3.10.3-3). Optimal growth appears to occur somewhere between 17.9 and 21.0 C (64.2 and 69.8 F). This is above the average annual temperatures found in Diablo Cove. This increase in growth rate with temperature is what Frank (1975) described as the major pattern of variation in Pacific Coast T. funebralis. Warmer water temperatures had the effect of decreasing the life span of T. funebralis, but it also increased their growth rate, decreased the time required to reach maturity, and increased their population biomass.

The both diet and temperature had significant effects on the growth of T. funebralis is of interest. The major effect of diet was due primarily to the preference of T. funebralis for brown algae, and their elevated consumption on

this diet. From the data it appears that the conversion efficiency (growth per unit consumption) is approximately the same on all diets. Since their digestive system can digest a large variety of algal nutrients (Galli and Giese 1959), it is likely that T. funebralis could exist on any available algae.

3.10.4 LAMINARIA DENTIGERA GAMETOPHYTE EARLY DEVELOPMENT AND GROWTH

Laminaria dentigera (oar weed or oar kelp), which is an important species at Diablo Canyon, occurs in the lower intertidal and upper subtidal (0-15 ft) zones at Diablo Canyon (Burge and Schultz 1973). The conspicuous plant in nature (the sporophyte) reproduces by the formation and release of microscopic zoospores, which settle, attach and develop into microscopic gametophytes. The gametophytes, which are male or female, undergo sexual reproduction to form new sporophytes. After zoospore production, the sporophyte will lose its blade but is capable of regenerating a new blade from the stipe. Survival of a population is therefore not entirely dependent on successful annual recruitment from gametophytes.

Included in this section are the results of two experiments on oar weed gametophytes. In the first, the effects of temperature and light intensity on zoospore germination and initial gametophyte development were studied. In the second, the influence of temperature and light intensity on growth and fertility of the gametophytes was determined.

The experiment described in this section involves the germination of zoospores and early development of gametophytes. The purpose was to provide information on the thermal response of L. dentigera populations. The objective was to determine the minimum, maximum and optimum temperatures for germination of zoospores and early development of gametophytes at several light levels.

3.10.4.1 LAMINARIA DENTIGERA GAMETOPHYTE EARLY DEVELOPMENT

The experiment described in this section involves the germination of zoospores and early development of gametophytes. The purpose was to provide information on the thermal response of L. dentigera populations. The objective was to determine the minimum, maximum and optimum temperatures for germination of zoospores and early development of gametophytes at several light levels.

Methods

A brief description of the methods used in this experiment is presented below. A more detailed description was presented in SECTION 3.7.1 (see also APPENDIX B, Procedure 309, and APPENDIX C, protocol for experiment 9c).

Fertile blades of L. dentigera were collected from a lower intertidal population in an area south of the power plant intake cove on 14 March 1980. They were transported to the laboratory in a plastic bag and held in ambient seawater. To induce spore release, several blades were scrubbed briefly in 0.2 percent bleach in seawater, rinsed in filtered seawater, dried at room temperature (20 C) for 10 minutes and then immersed in a beaker of 13 C seawater. The beaker was placed in a 13 C incubator. Spore release was sufficient after one hour to make a suspension for seeding the coverslips. Seeded coverslips were incubated at 13 C in the dark for six hours to allow the spores to settle and attach. Culture dishes were set up as for experiment 9a (see SECTION 3.7.1) at target temperatures of 9, 13, 17, 19, 21 and 25 C. Of the four dishes at each temperature, three were exposed to light intensities of 44, 77 and 110 $\mu E/m^2/s$, respectively, and one was maintained as a dark control.

One coverslip was removed from each dish and fixed in four percent formalin in seawater after 24, 48 and 72 hours. Percentage germination was assessed by observing 25 randomly selected fields of spores for each coverslip. To assess growth, 25 gametophytes were measured at the 72-hour sampling and again after one week.

Results

The temperature data and growth measurements are summarized in the following sections. Observations were made on germination of zoospores and measurements were made on resulting gametophyte filament lengths.

The mean, maximum and minimum temperatures recorded during the entire experiment for each probe are presented in TABLE 3.10.4-1. Heat given off by

TABLE 3.10.4-1

SUMMARY OF TEMPERATURE DATA:
LAMINARIA GAMETOPHYTE EARLY DEVELOPMENT

Target Temperature (C)	Target Light Intensity ($\mu E/m^2/s$)	Actual Temperature (C)				
		Mean	Standard Deviation	Maximum	Minimum	"Mean mean"
9	110	8.4	0.52	8.7	8.1	8.4
	77	8.6	0.30	9.0	8.2	
	44	8.4	0.50	8.7	8.2	
	0	8.2	0.51	8.4	8.0	
13	110	11.8	0.30	12.4	10.9	11.7
	77	11.8	0.30	12.4	11.0	
	44	11.6	0.28	12.2	11.0	
	0	11.8	0.59	12.4	11.1	
17	110	16.6	0.30	17.1	16.1	16.7
	77	16.7	0.28	17.1	16.2	
	44	16.6	0.26	17.0	16.1	
	0	16.7	0.30	17.2	16.2	
19	110	17.9	0.26	18.7	16.9	18.0
	77	18.1	0.59	19.0	17.1	
	44	18.1	0.50	18.9	17.2	
	0	18.0	0.51	18.8	17.1	
21	110	20.7	0.52	21.5	19.8	20.6
	77	20.5	0.30	21.3	19.7	
	44	20.7	0.50	21.5	19.8	
	0	20.6	0.51	21.4	19.8	
25	110	24.8	0.59	25.4	24.1	24.8
	77	24.8	0.28	25.4	24.1	
	44	25.1	0.30	25.5	24.3	
	0	24.6	0.52	25.3	23.9	

the artificial lights on the gradient apparatus affected the temperature of the seawater medium; thus, the maximum and minimum temperatures reflect the difference between daylight and night conditions. The mean value of the four mean temperatures for each light level is also shown ("mean mean") and is used in the following text in describing results.

There was no germination during the six-hour spore settlement period. The results of the 24-, 48- and 72-hour observations are shown in TABLE 3.10.4-2. Germination was completely inhibited at 24.8 C and will not be discussed further in this section. At the 24-hour sampling there is some evidence of possible synergism between light intensity and temperature (e.g., values for the 18.0 C treatment and for the 110 $\mu E/m^2/s$ treatment (FIGURE 3.10.4-1). Percentage germination generally increased in all treatments with time. A chi-square contingency test was applied to the 72 hour data to detect significant temperature effects (TABLE 3.10.4-3). There were no statistically diffferent values among the temperature treatments at 77 and 110 $\mu E/m^2/s$, but there were in the 0 and 44 $\mu E/m^2/s$ groups. Visual inspection of FIGURE 3.10.4-1 reveals that this most likely results from lower percentage germination values in the 20.6 C treatments of those two light levels (in fact the only decrease in percentage germination occurred between 48 and 72 hours in the 20.6 C treatments of the 0 and 44 $\mu E/m^2/s$ groups). Reorganization of the data to test for significant light intensity effects (TABLE 3.10.4-4) reveals essentially the same trend and further shows that light intensity had a significant effect at 18.0 C as well.

Since germination did not occur at 24.8 C, there are no growth data at that temperature. The average lengths of 25 gametophytes from the remaining conditions are presented in TABLE 3.10.4-5 and are plotted on a three-dimensional graph in FIGURE 3.10.4-2. After three days, filaments in the light conditions at 8.4, 11.7, 16.7 and 18.0 C are approximately equal in length. The 20.6 C and dark gametophytes are somewhat smaller.

Differences in size are more obvious after seven days. A two-way ANOVA performed on the average lengths showed significant effects on growth from both light and temperature and from the interaction of these two factors

TABLE 3.10.4-2

PERCENTAGE GERMINATION OF ZOOSPORES IN EXPERIMENT 9C:
LAMINARIA GAMETOPHYTE EARLY DEVELOPMENT

Sample Time (Hours)	Light Intensity ($\mu E/m^2/s$)	Treatment Temperature (C)					
		8.4	11.7	16.7	18.0	20.6	24.8
24	110	34.0	33.8	51.1	52.8	14.3	0
	77	31.2	55.3	50.0	44.4	18.4	0
	44	46.3	60.8	52.5	32.9	32.7	0
	dark	25.5	39.2	50.0	23.2	6.8	0
48	110	68.8	62.5	57.1	40.4	22.4	0
	77	56.0	41.2	60.0	34.5	38.6	0
	44	67.6	75.0	82.8	46.5	36.1	0
	dark	54.8	58.3	48.6	29.3	15.6	0
72	110	66.7	63.6	64.9	62.8	52.2	0
	77	57.4	55.3	76.7	59.1	42.5	0
	44	63.8	61.3	65.8	64.3	27.6	0
	dark	41.4	43.6	54.1	30.3	9.5	0

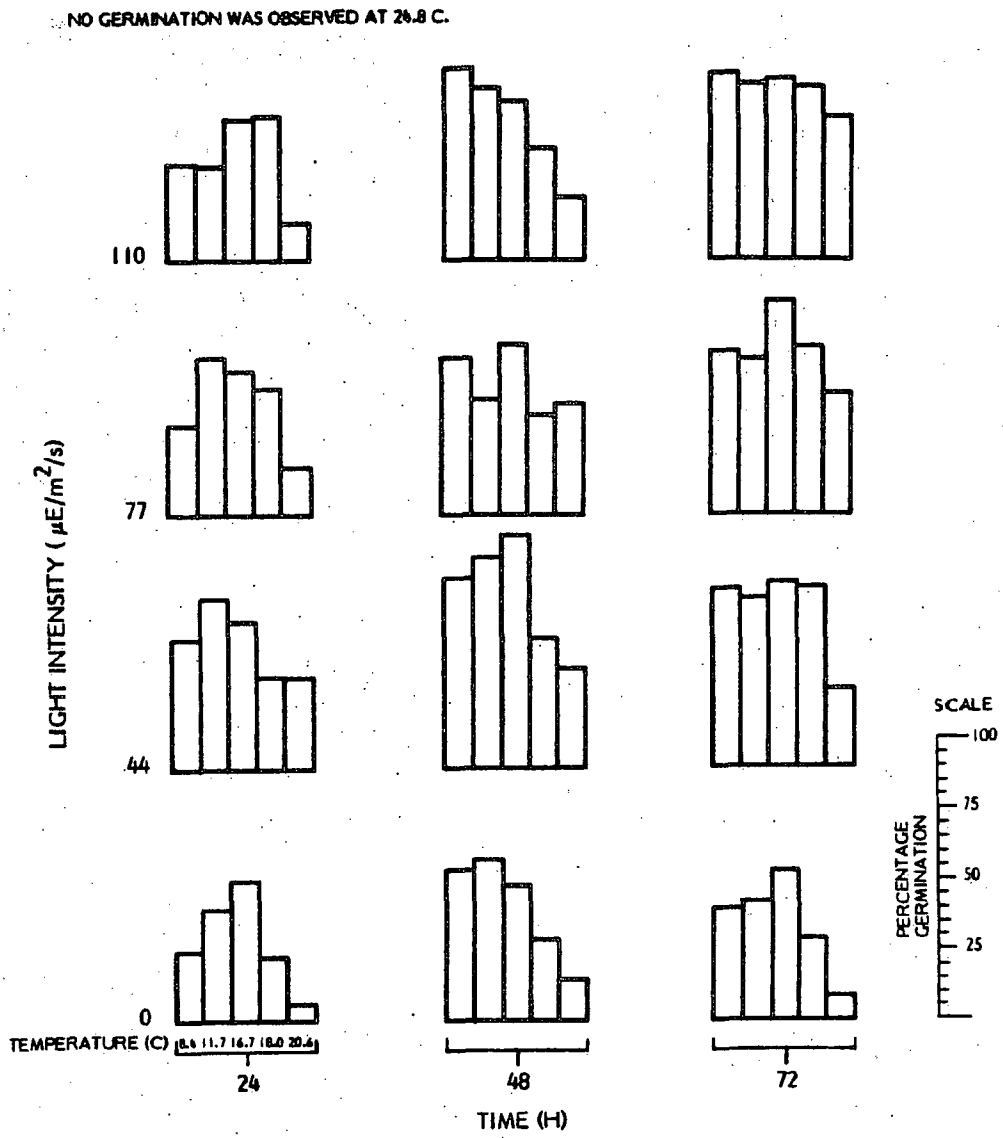


FIGURE 3.10.4-1

PERCENTAGE GERMINATION OF ZOOSPORES AT DAYS 1, 2 AND 3: LAMINARIA EARLY DEVELOPMENT

TABLE 3.10.4-3

CHI-SQUARE CONTINGENCY TABLE COMPARING ZOOSPORE GERMINATION WITH TEMPERATURE ON DAY 3: *LAMINARIA* GAMETOPHYTE EARLY DEVELOPMENT

x^2 =	Light Intensity ($\mu E/m^2/s$)		Temperature (C) 8.4	11.7	16.7	18.0	20.6	Sum
2.63	110	Germinated	38	28	24	27	24	141
		Ungerminated	19	16	13	16	22	86
		Sum	57	44	37	43	46	227
8.27	77	Germinated	27	21	23	26	17	114
		Ungerminated	20	17	7	18	23	85
		Sum	47	38	30	44	40	199
13.84*	44	Germinated	30	19	27	36	8	120
		Ungerminated	17	12	14	20	21	84
		Sum	47	31	41	56	29	204
13.83*	0	Germinated	24	17	20	20	2	83
		Ungerminated	34	22	17	46	19	138
		Sum	58	39	37	66	21	221

* Significant at p =0.01 (x^2 0.01,4df = 13.28).

TABLE 3.10.4-4

CHI-SQUARE CONTINGENCY TABLE COMPARING ZOOSPORE GERMINATION WITH LIGHT INTENSITY ON DAY 3: LAMINARIA GAMETOPHYTE EARLY DEVELOPMENT

χ^2 =	Temperature (C)		Light Intensity ($\mu E/m^2/s$) 110	77	44	0	Sum
8.77	8.4	Germinated	38	27	30	24	119
		Ungerminated	19	20	17	34	90
		Sum	57	47	47	58	209
3.85	11.7	Germinated	28	21	19	17	85
		Ungerminated	16	17	12	22	67
		Sum	44	38	31	39	152
3.79	16.7	Germinated	24	23	27	20	94
		Ungerminated	13	7	14	17	51
		Sum	37	30	41	37	145
18.75*#	18.0	Germinated	27	26	36	20	109
		Ungerminated	16	18	20	46	100
		Sum	43	44	56	66	209
12.93*@	20.6	Germinated	24	17	8	2	51
		Ungerminated	22	23	21	19	85
		Sum	46	40	29	21	136

* Significant at p = 0.01 (χ^2 0.01,3df = 11.34).

\# 0 $\mu E/m^2/s$ is lower than all others)

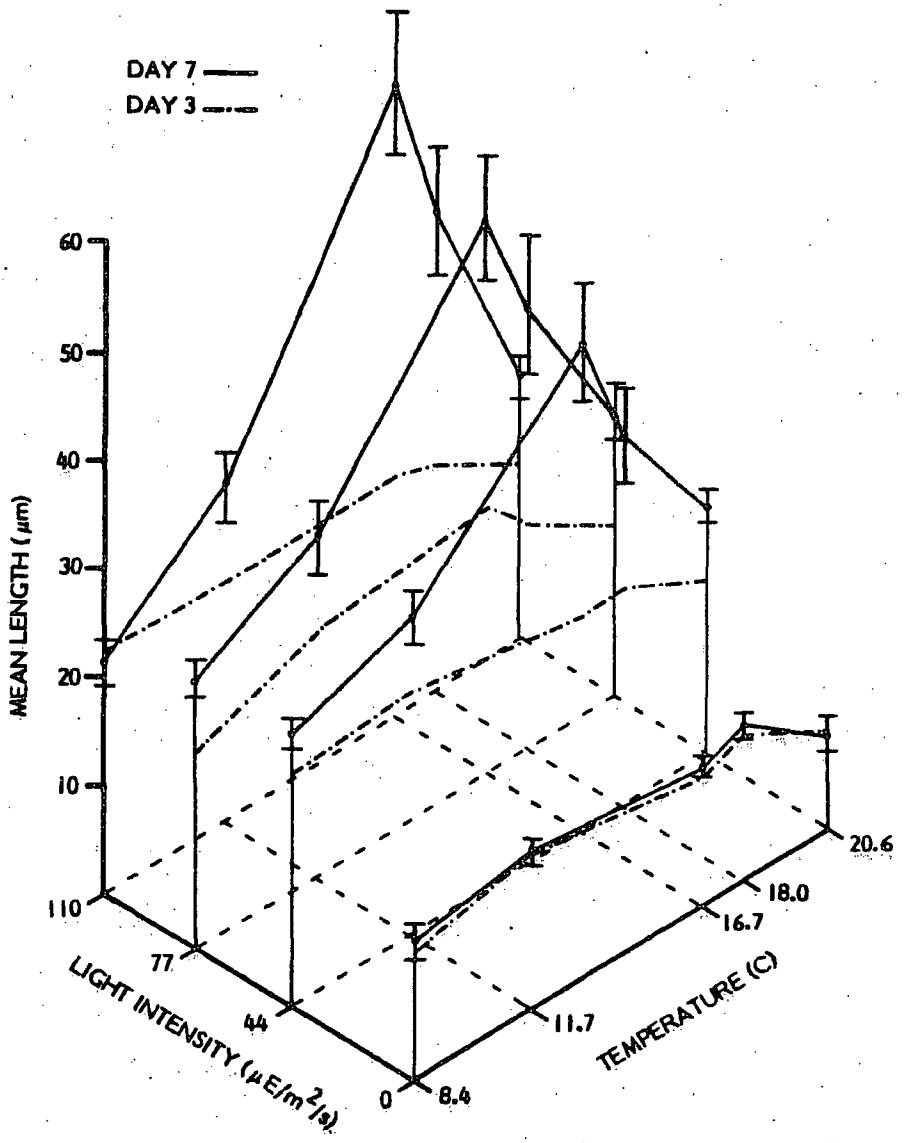
@ 0 $\mu E/m^2/s$ is lower than 77 and 110 $\mu E/m^2/s$.

TABLE 3.10.4-5

FILAMENT MEAN LENGTHS (μm) AND STANDARD DEVIATIONS (N = 25): *LAMINARIA* GAMETOPHYTE EARLY DEVELOPMENT

Sample Period (Days)	Light Intensity ($\mu E/m^2/s$)	Temperature (C)				
		8.4	11.7	16.7	18.0	20.6
3	110	22.3 (5.78)	21.9 (4.04)	22.7 (5.40)	21.0 (4.81)	16.2 (3.82)
	77	17.9 (4.80)	22.3 (3.93)	24.3 (5.78)	20.8 (5.01)	15.6 (4.01)
	44	21.6 (4.13)	22.1 (4.04)	20.0 (4.42)	20.0 (5.23)	16.2 (3.47)
	0	11.8 (4.65)	14.3 (3.62)	12.0 (3.33)	13.2 (2.72)	8.9 (1.42)[a]
7	110	21.3 (5.32)	30.9 (8.37)	58.8 (17.08)	44.5 (14.49)	24.3 (5.06)
	77	24.8 (4.45)	31.4 (8.92)	51.5 (14.71)	41.2 (16.00)	26.4 (6.69)
	44	25.1 (4.20)	29.3 (6.40)	45.7 (14.89)	34.6 (11.61)	22.9 (4.10)
	0	13.0 (4.19)	14.7 (3.26)	12.8 (2.46)	14.1 (2.73)	8.7 (2.4)[a]

[a] N = 10



Vertical bars represent 95 percent confidence limits
(N = 25 except N = 10 for 20.6 C, dark treatment).

FIGURE 3.10.4-2

MEAN FILAMENT LENGTH VERSUS TEMPERATURE AND LIGHT INTENSITY AT DAYS 3 AND 7: LAMINARIA GAMETOPHYTE EARLY DEVELOPMENT

(TABLE 3.10.4-6). Differences between treatment means (except the 20.6 C dark treatment because the N was only 10) were tested using the method of least significant difference (LSD). The results of this test are shown in FIGURE 3.10.4-3. At 16.7 C growth was optimal at 110 $\mu E/m^2/s$ with a significant decrease at 77 and 44 $\mu E/m^2/s$. At 18.0 C there was a significant drop in growth at 44 $\mu E/m^2/s$, whereas at 20.6, 11.7 and 8.4 C there were no significant light intensity effects. Growth in the dark was significantly lower at all temperatures.

Peak development occurred at 16.7 C and the decrease in growth from this temperature to 11.7 C was significant at all light levels. This was also true for the decrease in growth from 18.0 to 20.6 C. There was some overlap between 16.7 and 18.0 C, but only at the lowest light level in 16.7 C. Temperature had no effect on growth in the dark cultures.

Discussion

In petri dish culture, germination rates are optimal in all light conditions from 8.4 to 18.0 C (47 to 64 F). Significant decreases in rate occur in dark samples at 18.0 and 20.6 C (64 and 69 F). Zoospores would probably never be exposed to continuous darkness in nature, but could be subjected to light levels lower than 44 $\mu E/m^2/s$ (i.e., 0-44 $\mu E/m^2/s$). At temperatures between 18.0 and 20.6 C (64 and 69 F) this condition could inhibit germination significantly. Germination at or above 24.8 C (76.6 F) would be totally inhibited, regardless of the light intensity (in a range from 0-110 $\mu E/m^2/s$). There are no references to temperature effects on germination for *L. dentigera* in the literature. Hsiao and Druehl (1971) reported a slight decrease in germination in the dark at 10 C (50 F) for *L. saccharina*.

3.10.4.2 *LAMINARIA DENTIGERA* GAMETOPHYTE GROWTH AND FERTILITY

The experiment described in this section is an abbreviated version of the optimum temperature for growth and fertility procedure. The purpose of the

TABLE 3.10.4-6

TWO-WAY ANALYSIS OF VARIANCE OF FILAMENT LENGTH AT DAY 7: *LAMINARIA* GAMETOPHYTE EARLY DEVELOPMENT

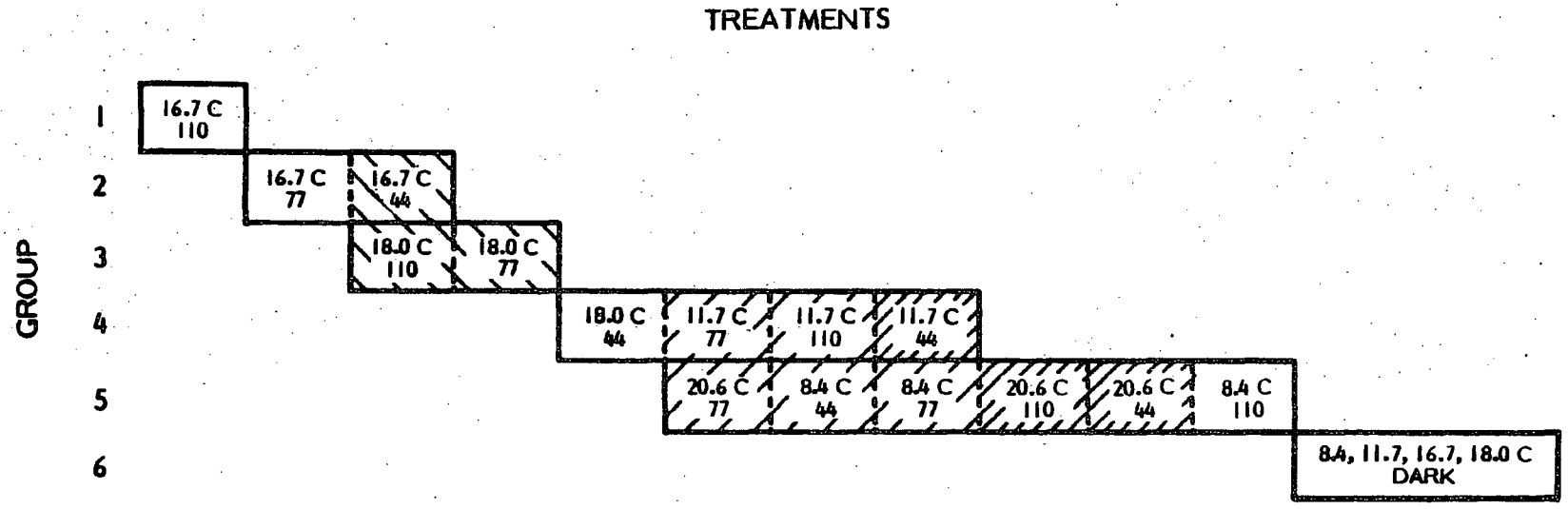
Light Intensity ($\mu E/m^2/s$)	Temperature (C)					
	8.4	11.7	16.7	18.0	20.6	Sum
0	325	367.5	320	352.5	217.5	1,582.5
44	627.5	732.5	1,142.5	865	572.5	3,940
77	619.1	785	1,287.5	1,030	660	4,381.6
110	557.5	772.5	1,470	1,112.5	607.5	4,520
Sum	2,129.1	2,657.5	4,220	3,360	2,057.5	14,424.1

Sum x^2 = 546,453.4

Source	df	SS	MS	F_{comp}	$F_{.99}$
Light	3	35,007.09	11,669.03	144.93	3.78
Temperature	4	27,758.65	6,939.66	86.19	3.32
Interaction	12	17,269.93	1,439.16	17.87	2.18
Error	465	37,439.05	80.51		
Total	484	117,474.72			

$$LSD = t_{0.01,\,00} \cdot \sqrt{\frac{2}{N} : MSE} = 2.576 \sqrt{\frac{2}{25} 80.51} = 6.54$$

Numbers in squares are temperature values (C, top) and light values ($\mu E/m^2/s$, bottom). Mean lengths decrease from left to right and from top to bottom. Members of groups are statistically the same (P = 0.01).



Groups 1 and 6 are distinct, Group 2 overlaps 3 (16.7 C, 44 into 3)

Groups 3 and 4 do not overlap

Group 4 combines with Group 5 in the following way:

- 18.0 C, 44 does not overlap
- 11.7 C, 77 and 110 overlap through 8.4 C, 77
- 11.7 C, 44 overlaps through 20.6 C, 44
- 8.4 C, 110 does not overlap with Group 4 members

FIGURE 3.10.4-3

RESULTS OF LSD TEST ON MEAN LENGTHS OF DAY 7:
LAMINARIA GAMETOPHYTE EARLY DEVELOPMENT

experiment was to provide information on the thermal tolerance of L. dentigera. The objective was to assess fertility of established gametophytes and subsequent sporophyte production at two light intensities (110 and 165 $\mu E/m^2/s$) and five temperatures (9, 13, 16, 18 and 21 C). SECTION 4.3.4 of this report presents the results of an experiment on gametophyte early development (germination and initial growth of zoospores).

Methods

The procedures used for this experiment were the same as for the bull kelp fertility experiment (see SECTION 3.7.2 and APPENDIX B, Procedure 318) with the omission of growth measurements. The protocol for this experiment (18c) is included in APPENDIX C. The fertile plants were collected on 18 April 1980 from a low intertidal area south of the power plant intake cove. Spore release, settlement and initiation of the experiment were carried out as previously described. Gametophyte cultures were observed for fertility on days 3, 7 and 10.

Results

A summary of the temperature data for the entire experiment is presented in TABLE 3.10.4-7. The mean value of the two means at each temperature is used in the following text in describing results.

The percentage of fertile males and females along with the mean number of eggs per fertile female at days 7 and 10 are shown in TABLE 3.10.4-8. No fertility was observed at day 3. Male fertility at day 7 was high (80 percent or higher) in all but the 17.9 C cultures. By day 10, male fertility was at as near 100 percent in all cultures (FIGURE 3.10.4-4) (the 17.9 C, 165 $\mu E/m^2/s$ treatment could not be sampled at day 10). Both female fertility and sporophyte production at day 7 were highest at 12.8 and 15.4 C. This trend continued to day 10. Sporophytes were first observed in the 10.0 C cultures on day 10; however, they were mostly 2-4 celled. Sporophytes at both 12.3 and 15.4 C were larger and in most cases beginning to form rhizoids. No sporophytes were observed at 17.9 C during the experiment (TABLE 3.10.4-9).

TABLE 3.10.4-7

SUMMARY OF TEMPERATURE DATA:
LAMINARIA GAMETOPHYTE GROWTH AND FERTILITY

Target Temperature (C)	Light Intensity ($\mu E/m^2/s$)	Actual Temperature (C)				Combined Mean
		Mean	Standard deviation	Maximum	Minimum	
9	110	10.1	0.32	10.8	9.4	10.0
	165	9.9	0.28	10.5	9.3	
13	110	12.8	0.46	13.5	12.0	12.8
	165	12.9	0.56	13.6	11.9	
16	110	15.3	0.70	16.6	14.0	15.4
	165	15.5	0.74	16.8	14.2	
18	110	17.9	0.87	19.3	16.2	17.9
	165	18.0	0.90	19.4	16.3	
21	110	20.1	0.32	21.7	18.5	20.1
	165	20.1	0.28	21.2	18.4	

TABLE 3.10.4-8

FERTILITY AT DAYS 7 AND 10:
LAMINARIA GAMETOPHYTE GROWTH AND FERTILITY

Temperature (C)	Light Intensity ($\mu Em^{-2}s^{-1}$)	Day 7			Day 10		
		Percentage Fertile Females	$\overline{X}$ No. eggs/female*	Percentage Fertile Males	Percentage Fertile Females	$\overline{X}$ No. eggs/female*	Percentage Fertile Males
10.0	165	4	1.0 (0.0)	100	14	1.0 (0.0)	100
	100	2	1.0 (0.0)	88	18	1.0 (0.0)	98
12.8	165	78	1.0 (0.0)	100	40	1.1 (0.4)	100
	110	32	1.1 (0.4)	100	82	1.0 (0.0)	100
15.4	165	64	1.2 (0.6)	98	98	1.6 (0.7)	100
	110	48	1.2 (0.4)	100	52	1.1 (0.4)	100
17.9	165	16	1.3 (0.5)	16	-- no observations --		
	110	20	1.4 (0.5)	56	36	1.4 (0.6)	100
20.1	165	0	—	0	0	--	0
	110	0	—	0	0	--	0

* Standard deviation in parentheses.

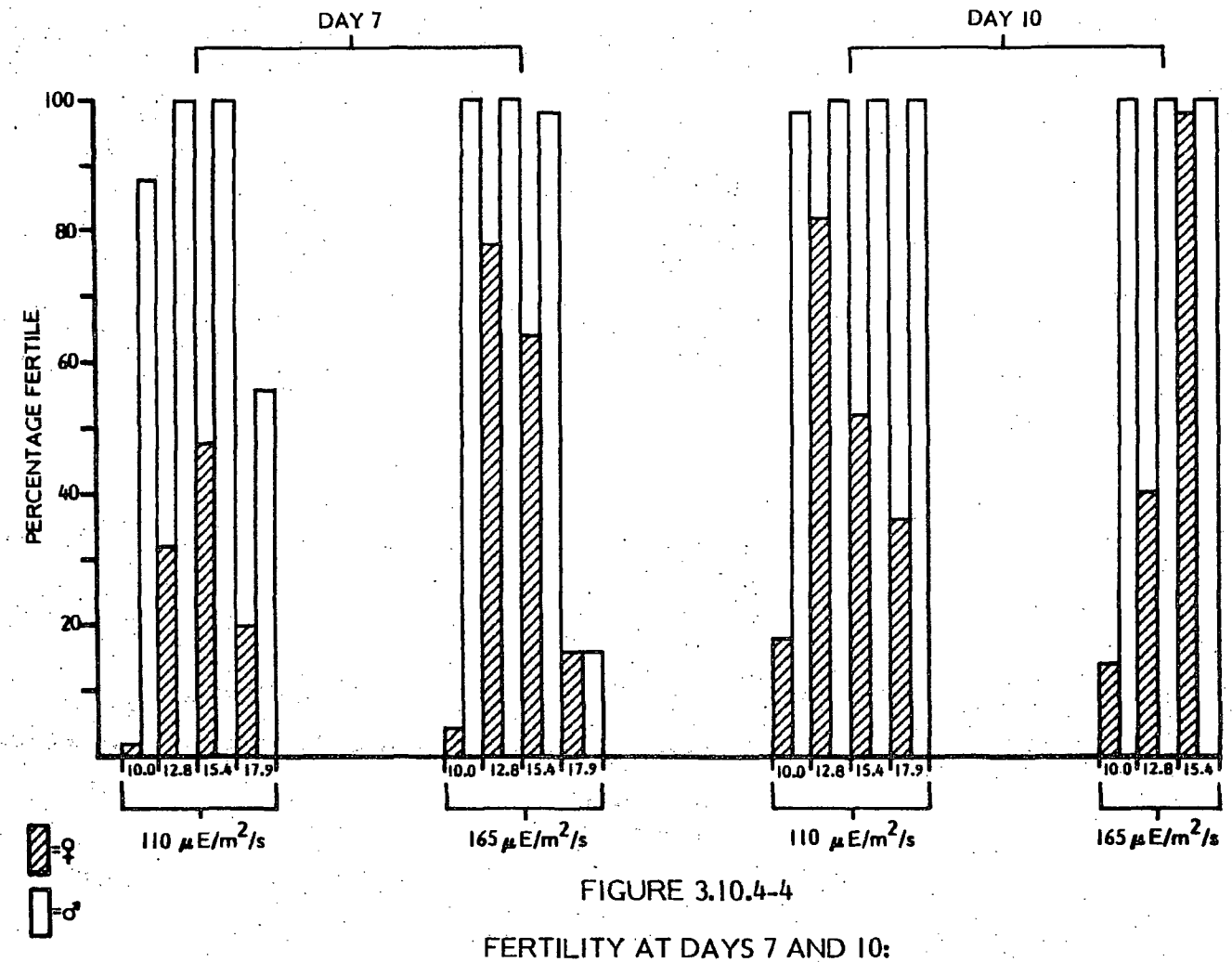


FIGURE 3.10.4-4

FERTILITY AT DAYS 7 AND 10:
LAMINARIA GAMETOPHYTE GROWTH AND FERTILITY

TABLE 3.10.4-9

SPOROPHYTE PRODUCTION AT DAYS 7 AND 10:
LAMINARIA GAMETOPHYTE GROWTH AND FERTILITY

Temperature (C)	Light Intensity ($\mu E/m^2/s$)	Day 7			Day 10		
		Total No. Females w/Sporophytes	Total No. Sporophytes	$\bar{X}$ Sporophytes/Female	Total No. Females w/Sporophytes	Total No. Sporophytes	$\bar{X}$ Sporophytes/Female
10.0	165	0	--	--	5	5	1.0
	110	0	--	--	4	4	1.0
12.8	165	30	36	1.2	13	18	1.4
	110	9	10	1.1	41	48	1.2
15.4	165	11	12	1.1	38	65	1.7
	110	7	10	1.4	21	26	1.2
17.9	165	0	--	--	0	--	--
	110	0	--	--	0	--	--

Discussion

Fertility of both male and female Laminaria dentigera gametophytes was totally inhibited at 20.1 C (68.2 F). Male fertility seemed to be unaffected in a range of temperatures from 10.0 to 17.9 C (50 to 64.2 F). Female fertility was high at 12.8 and 15.4 C (55 and 59.7 F) in terms of both the number of fertile females and the number of sporophytes produced by them. The peak values for these characteristics occurred at 15.4 C (59.7 F), 165 $\mu E/m^2/s$. Female fertility was considerably lower at 10.0 C (50 F) but a few sporophytes were observed during the course of the experiment. At 17.9 C (64.2 F), female fertility was higher than at 10 C (50 F), but no sporophytes were observed.

In terms of peak female fertility, the results of this experiment agree with those of Lüning and Neushul (1978) who observed optimum female fertility at 12 C (53.6 F) for Laminaria dentigera. The upper limits for reproduction differ in that Lüning and Neushul observed total inhibition of female fertility at 17 C (62.3 F), after two weeks in culture. This upper limit for fertility was common for all of the central California species tested by them. For this experiment, fertile females were evident at 17.9 C (64.2 F), with total inhibition at 20.1 C (68.2 F). Lüning and Neushul did observe an upper limit for female fertility at 20 C for southern California (Santa Barbara) species. It appears that the Laminaria from the Diablo Canyon area has a temperature response more in common with southern California Laminariales.

3.10.5 BOTRYOGLOSSUM FARLOWIANUM GROWTH

Botryoglossum farlowianum is one of the most abundant of the shrubby understory red algae in the shallow subtidal communities of Diablo Cove. For this reason, an experiment was carried out to determine the optimum temperature for growth of this species.

Geographically, Botryoglossum farlowianum is distributed from Vancouver I., British Columbia, Canada to Baja California, Mexico (Abbott and Hollenberg 1976). Two varieties of the species are recognized: B. farlowianum var. farlowianum and B. farlowianum var. anomalum. The criterion for distinction between the two is the position of the tetrasporangial sori along:

- Blade margins, or
- Terminal veins.

Determination of variety is therefore possible only for tetrasporangial specimens; all blades employed in this study were non-reproductive and no attempt was made to identify variety. B. farlowianum var. farlowianum is, however, the more widely distributed of the two, occurring throughout the entire range reported for the species, while B. farlowianum var. anomalum occurs only from Marin County south to San Luis Obispo County in California (Abbott and Hollenberg 1976).

Botryoglossum presumably undergoes a typical red algal triphasic life history, with both gametophytes and tetrasporophytes being conspicuous upright plants of similar morphology. Numerous blades may be produced along the stoloniferous basal system (see also PGandE 1979b).

Information concerning ecological and physiological characteristics of Botryoglossum is noticeably lacking in the literature. A heat tolerance experiment performed in April 1979 (see SECTION 2.10.28) provided information useful in determining test temperatures to be employed in long-term growth experiments. In the cited report, temperatures of 23.8 C were shown to be lethal, and

temperatures of 19.1 and 21.0 C were suggested to be sublethal. The maximum temperature to be used in this growth experiment was therefore chosen as 20 C. Other temperature treatments were designed at decreasing stepwise intervals of 2 C (18, 16, 14 and 12 C). No ambient seawater temperature treatment was employed.

In comparing results and conclusions of the previously reported Botryoglossum heat tolerance experiment with the Botryoglossum growth experiment presented here, it is very important to note differences in procedure which may have affected the implied thermal heat tolerance of the alga. No "temperature adjustment" period was provided in the heat tolerance experiment, the plants having been initially transferred from an ambient seawater temperature of about 12 C directly to treatment temperatures. Thus 'heat shock' effects probably contributed to the thermal effects at higher experimental temperatures. Temperature adjustment was carried out prior to initiation of the growth experiment reported here in an attempt to minimize heat shock effects. Of importance also is the difference in photoperiod used in the two experiments. The heat tolerance experiment was conducted under continuous (24 hours/day) illumination, whereas the growth experiment was conducted with a 16:8 (hours light:hours dark) cycle.

Material used in the heat tolerance experiment was larger in size and more densely branched on average than that selected for the growth experiment. Additionally, for the purposes of the heat tolerance experiment, individual plants were clipped onto semi-stationary PVC racks maintained within the raceways, whereas culture conditions of the growth experiment employed aquaria provided with auxillary pumps to ensure constant agitation of the experimental plants (see methods below).

The objective of the growth experiment was to determine the temperature promoting maximum growth of B. farlowianum in the laboratory, in order that effects of elevated water temperatures in Diablo Cove on the species populations as revealed by field studies could be more accurately interpreted.

3.10.5.1 METHODS

Culture and measurement techniques performed during the experiment are summarized below. For a more detailed description of experimental procedure see APPENDIX B (Procedure 325) and APPENDIX C (experiment protocols 25a and 25b). For a description of the raceway and seawater systems used, refer to APPENDIX A.

Two runs of the Botryoglossum growth experiment were made. The first of these incorporated five temperature treatments (12, 13, 16, 18 and 20 C), of which only four (12, 14, 16, and 18 C) were carried to completion. Inadequate temperature control forced premature termination of the 20 C treatment at experimental day 14. The second run of the experiment employed only two temperature treatments: a 20 C treatment was initiated to supplement incomplete data from the aborted first run, and a 16 C treatment was incorporated to provide a relative basis of comparison between the two runs. The first and second runs are referred to, respectively, as 25a and 25b, to allow for the reader's distinction between the two. Data from each run are treated separately, and are followed by a summary discussion and comparison of all experimental results.

RUN 25a. Small non-reproductive blades of B. farlowianum were collected by divers on 1 February 1980, from depths of 15-20 ft in north and central Diablo Cove. In the laboratory, the plants were held pre-experimentally for a period of five days in raceways provided with flowing ambient seawater. Irradiance during this time, and for the entire experimental period, was provided by overhead banks of 25W Duro Test Ultra-High Output fluorescent bulbs. A 16:8 (hours light:hours dark) photoregime was employed. During the pre-experimental period plants were sorted into experimental, or 'treatment', groups of 20 blades each. Blades were selectively distributed among treatment groups so that initial total wet weight of each group was roughly comparable to that of the others. Initial wet weight values are presented in TABLE 3.10.5-1. Plants with visible epiphytes were excluded from experimental groups.

TABLE 3.10.5-1

INITIAL TOTAL WET WEIGHT (G) FOR EACH EXPERIMENTAL TEMPERATURE TREATMENT (N = 20): BOTRYOGLOSSUM GROWTH

Treatment Temperature (C)	12	14	16	18	20
Run 25a Initial Wet Weight (G)	44.0	46.3	45.8	43.0	48.1

Treatment Temperature (C)	16	20
Run 25b Initial Wet Weight (G)	31.8	30.2

Transfer of plants to designated experimental temperatures was preceded by a temperature adjustment period of at least four hours per each 2 C increase or decrease from ambient seawater temperatures. Each group of 20 plants was introduced into one of five experimental treatments. Each was maintained throughout the experiment within a closed 5 gallon glass aquarium with an auxilliary pump providing non-recirculated seawater at approximately 270 GPH. The entire aquarium system was immersed in a raceway filled with seawater of the desired experimental temperature. The raceway water thereby functioned as both a temperature-controlled water bath and as a source of fresh seawater supplied to the aquarium via the auxillary pump. For a more detailed description of the aquarium culture system, refer to APPENDIX B (Procedure 325). Each group of plants was weighed (wet) at seven-day intervals for a period of 28 days.

Light intensity in each raceway was measured twice during the experiment, using a Li-Cor Quantum Meter equipped with an underwater sensor held at the upper edge of the aquarium (approximately 25 cm below the raceway water surface). Water samples were collected weekly from each experimental raceway. These were subsequently analyzed for pH, salinity, nitrate, nitrite, ammonia, and phosphate concentrations.

Raceways and aquaria were cleaned periodically as described in the Experiment Procedure (see APPENDIX B).

Water temperatures within the raceways and aquaria were maintained and recorded hourly by the Autodata Nine computer. A single temperature probe was positioned within each aquarium, passing through a hole in the aquarium lid.

RUN 25b. Small non-reproductive blades of *B. farlowianum* were collected by divers on 10 March 1980, from depths of 15-25 ft in north and central Diablo Cove. The plants were treated as described for run 25a above, with the exception that plants were held pre-experimentally for two rather than five days. Initial wet weights of treatment groups are presented in TABLE 3.10.5-1. Experimental treatments were maintained at target temperatures of 16 and 20 C. All measurements were made according to procedures outlined for run 25a above.

3.10.5.2 RESULTS

Because of the differences in methods used in the two runs, data from each are considered separately in the two subsections below.

RUN 25a. A summary table of experimental temperatures is presented in TABLE 3.10.5-2. Mean and standard deviation values are given for each group, as well as maximum and minimum temperatures recorded within each treatment. All values refer to the entire 28-day experimental period, with the exception of those pertaining to the 20 C treatment, which extended only to 14 days. In every case, treatment temperature was maintained within $\pm$ 1 C of the target temperature. The temperature variation about the mean is in part attributable to daytime heating of raceway water by the overhead lights, and nighttime cooling while the lights were shut off. The 12, 18, and 20 C treatments experienced the greatest range of temperature variation (1.3 C); the 14 C treatment range was 1.1 C, and the 16 C treatment, 0.8 C.

Light intensity measurements are reported in TABLE 3.10.5-3. Values are given in $\mu E/m^2/s$; each represents an instantaneous single point reading. Measured intensities ranged from a minimum of 111 $\mu E/m^2/s$ for the 12 C treatment to a maximum of 140 $\mu E/m^2/s$ for the 18 C treatment.

Results of seawater nutrient analyses are presented in TABLE 3.10.5-4. In all samples, ammonia concentrations were below levels of detectability at <0.3 $\mu g/l$ (micrograms per liter). Nitrite concentrations were below detectability levels of <3.0 $\mu g/l$ at the first, fourth, and fifth sample periods; at the second and third sample periods, nitrite concentrations ranged from <3.0 to 13 $\mu g/l$. Nitrate concentrations remained, at the first three sample periods, at or below detectability levels of 10 $\mu g/l$; samples taken during the last two periods exhibited an increase in nitrate concentrations, up to 30 $\mu g/l$. Phosphate concentrations at five sampling times ranged from a maximum of 40 $\mu g/l$ to a minimum of 10 $\mu g/l$ (below detectability). The pH values of the samples taken ranged from a minimum of 7.7 to a maximum of 8.1. Salinity varied from 32-34 ppt (parts per thousand) over all samples taken.

TABLE 3.10.5-2

STATISTICAL SUMMARY OF TEMPERATURE DATA (RUN 25a): BOTRYOGLOSSUM GROWTH

Target Temperatures (C)	Actual Temperatures			
	Mean	Standard Deviation	Maximum	Minimum
12	12.0	0.11	12.9	11.6
14	14.2	0.16	14.8	13.7
16	16.1	0.15	16.5	15.7
18	18.0	0.17	18.5	17.2
20	20.1	0.13	20.7	19.4

TABLE 3.10.5-3

LIGHT INTENSITY VALUES (IN $\mu E/m^2/s$) FOR EACH TEMPERATURE TREATMENT AT TWO MEASUREMENT TIMES (RUN 25a): BOTRYOGLOSSUM GROWTH

Date	Treatment (C)				
	12	14	16	18	20
13 February	116*	132	143	138	121
22 February	111	122	136	140	121

*measurement made on 15 February.

TABLE 3.10.5-4

RESULTS OF WATER QUALITY ANALYSES FOR RUN 25a: BOTRYOGLOSSUM FOR GROWTH

Units are as follows: $\mu g/l$ for NH_3 (ammonia), NO_2 (nitrite), NO_3 (nitrate), and PO_4 (phosphate); parts per thousand for salinity.

Sample Date	Target Temperature (C)	Parameter					
		pH	NH_3	NO_2	NO_3	PO_4	Salinity
7 February	12	7.7	0.3	3.0	10	30	33
	14	7.7	0.3	3.0	10	15	33
	16	7.7	0.3	3.0	10	15	33
	18	7.7	0.3	3.0	10	15	33
	20	7.7	0.3	3.0	10	40	33
14 February	12	8.0	0.3	3.0	10	15	33
	14	8.0	0.3	11.0	10	15	32
	16	8.0	0.3	3.0	10	15	32
	18	7.9	0.3	13.0	10	30	32
	20	7.9	0.3	3.0	10	15	32
21 February	12	7.9	0.3	11.0	10	40	32
	14	7.9	0.3	9.0	10	30	32
	16	7.9	0.3	6.0	10	40	32
	18	7.9	0.3	13.0	10	30	32
	20	7.9	0.3	9.0	10	40	32
28 February	12	8.0	0.3	3.0	30	10	32
	14	8.0	0.3	3.0	20	20	32
	16	8.0	0.3	3.0	20	10	32
	18	8.1	0.3	3.0	30	10	32
6 March	12	8.0	0.3	3.0	10	10	32
	14	8.0	0.3	3.0	10	10	33
	16	8.0	0.3	3.0	20	10	34
	18	8.0	0.3	3.0	10	10	33

Actual increase in wet weight of plants within each treatment group is plotted in FIGURE 3.10.5-1. Values represent total combined wet weight of all plants within a single treatment, and therefore no confidence interval bars are shown. By day 28, 16 C had clearly emerged as the treatment temperature promoting greatest increase in plant wet weight. A sharp decline in wet weight increase occurred between the 16 and 18 C treatment groups by day 28. FIGURE 3.10.5-2 shows wet weight increase through time, plotted on a semilog scale. The log transferred data for all remaining treatments show a linear function through time with nearby identical slopes. The 20 C treatment was discontinued after day 14 because of temperature variations in the tank.

RUN 25b. A summary of experimental temperatures for run 25b is given in TABLE 3.10.5-5A. Mean and standard deviation values are given for each group, as well as maximum and minimum temperatures experienced within each treatment. All values are derived from the entire 28 day experimental period. The 16 C treatment averaged 16.2 C, with a maximum temperature range of 1.5 C. The 20 C treatment averaged 19.8 C, with a range of 1.2 C. As noted for run 25a, temperature variation about the mean is in part attributable to daytime heating of the raceway water by the overhead lights, and the accompanying nighttime cooling of the same. Measured light intensities are presented in TABLE 3.10.5-5B. Values represent an instantaneous, single-point reading. Measured intensities varied from 149 to 154 $\mu E/m^2/s$. Results of seawater nutrient analyses are presented in TABLE 3.10.5-5C. In all samples taken, ammonia concentrations remained below levels of detectability at 0.3 g/l. Similarly, nitrite concentrations in all samples were below detectability levels of $< 3.0\ \mu g/l$. Nitrate concentrations in both samples of the first period were 20 $\mu g/l$; at the second sample period, nitrate concentrations had risen to 60-70 $\mu g/l$. Phosphate concentrations among samples ranged from 10 to 40 $\mu g/l$. For all samples pH was measured at 7.7. Salinity was 33 parts per thousand in all samples.

Total combined (all plants in each treatment) wet weights through time for both treatments is plotted in FIGURE 3.10.5-3. Plants maintained at 16 C exhibited consistently greater increases in wet weight through time than did plants grown

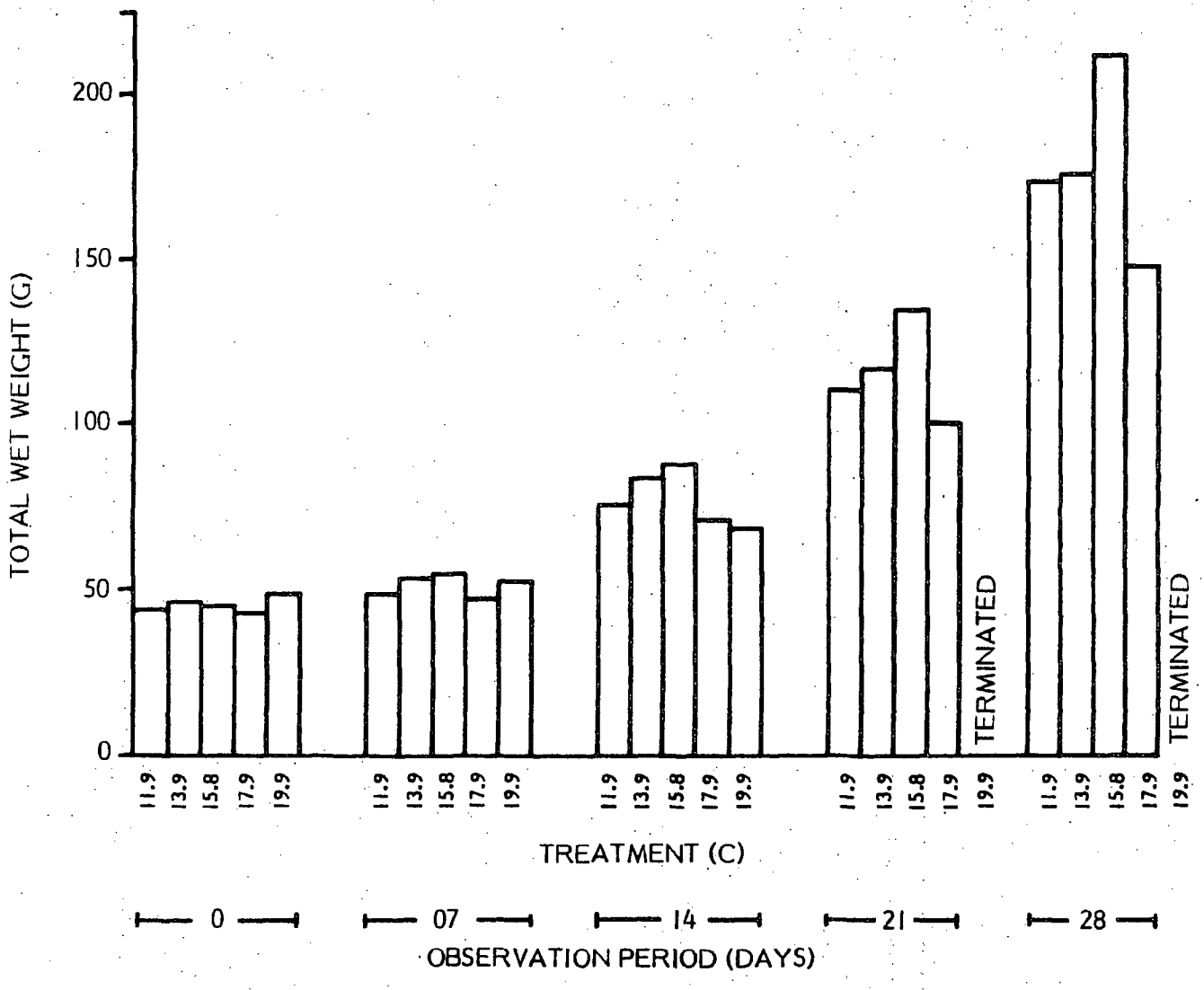


FIGURE 3.10.5-1

WET WEIGHT VERSUS TIME FOR RUN 25A:
BOTRYOGLOSSUM GROWTH

Lines fitted by eye to last four observation points. 20C treatment discontinued after day 14. Values are total wet weight for all plants in each treatment.

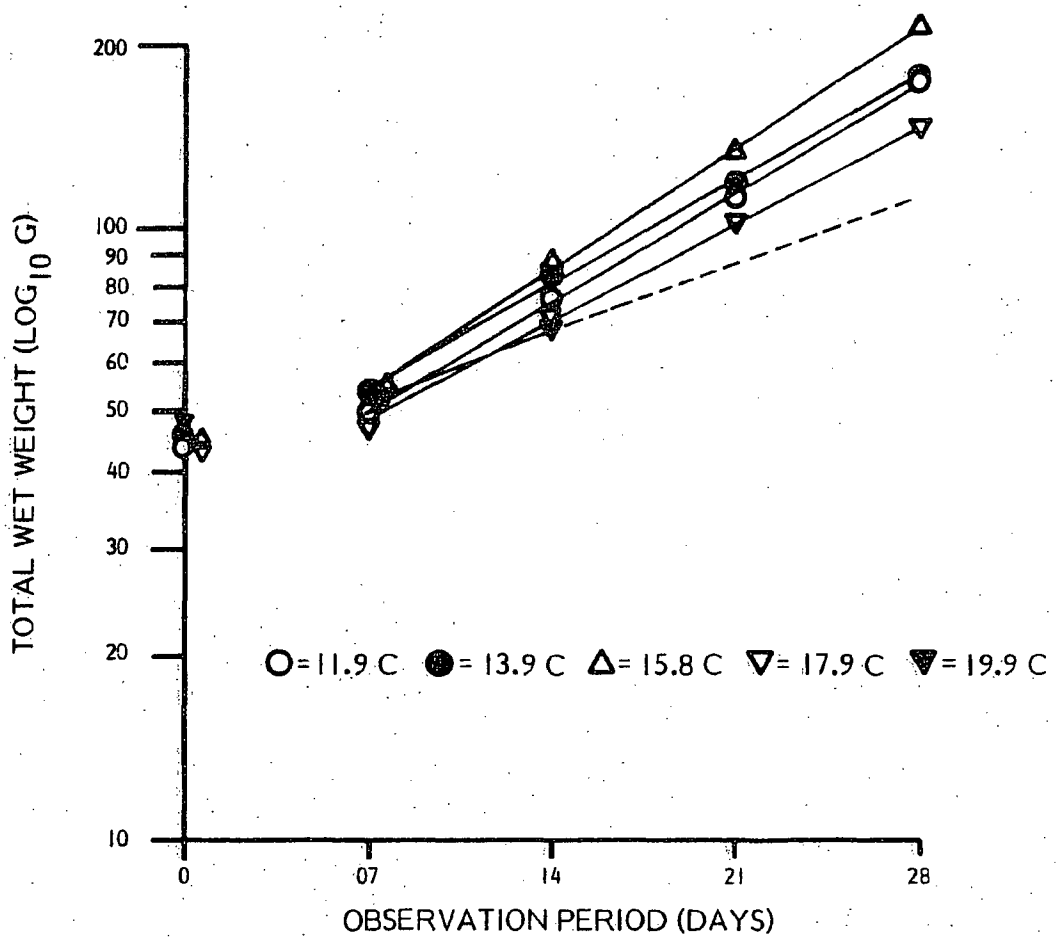


FIGURE 3.10.5-2

WET WEIGHT VERSUS TIME FOR RUN 25A:
BOTRYOGLOSSUM GROWTH RATE

TABLE 3.10.5-5

PHYSICAL AND CHEMICAL MEASUREMENTS TAKEN IN RUN 25b: BOTRYOGLOSSUM FOR GROWTH

A. TEMPERATURE DATA

Target Temperature (C)	Actual Temperature (C)			
	Mean	Standard Deviation	Maximum	Minimum
16	16.2	0.13	16.4	15.9
20	19.8	0.10	20.0	18.8

B. LIGHT INTENSITY DATA ($\mu E/m^2/s$)

Date	Treatment (C)	
	16	20
28 March	154	149
2 April	154	154

TABLE 3.10.5-5 (Cont.)

C. WATER QUALITY DATA

Units are as follows: $\mu g/l$ for NH_3 (ammonia), NO_2 (nitrite), NO_3 (nitrate), and PO_4 (phosphate); parts per thousand for salinity.

Sample Date	Target Temperature (C)	Parameter					
		pH	NH_3	NO_2	NO_3	PO_4	Salinity
28 March	16	7.7	0.3	3.0	20	10	33
	20	7.7	0.3	3.0	20	20	33
2 April	16	7.7	0.3	3.0	70	20	33
	20	7.7	0.3	3.0	60	40	33

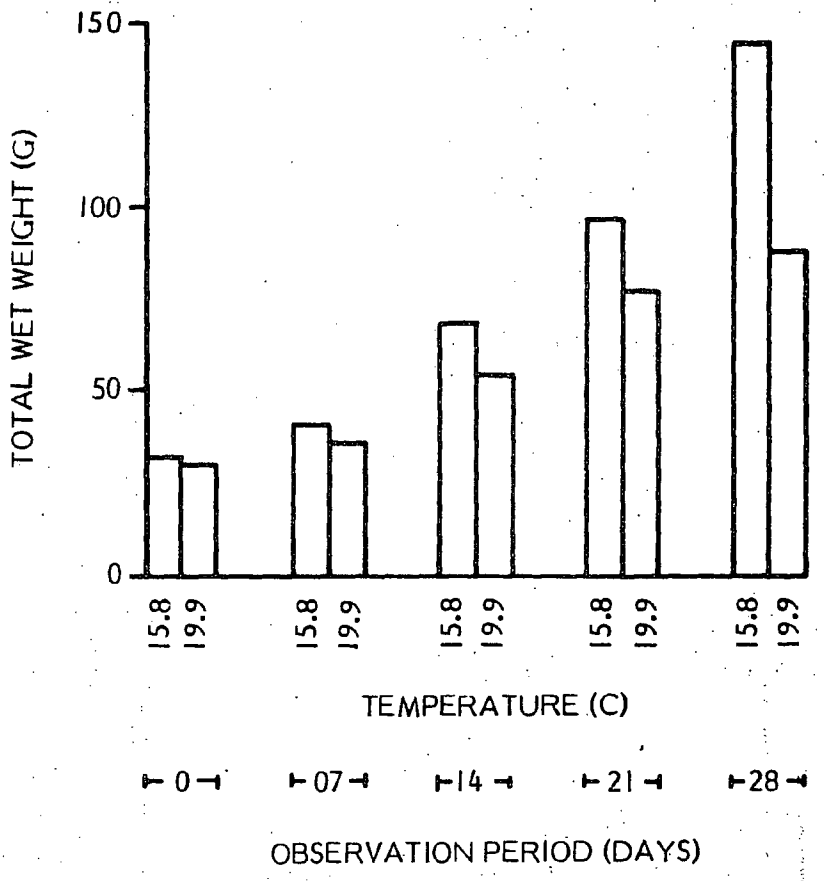


FIGURE 3.10.5-3

WET WEIGHT VERSUS TIME FOR RUN 25B:
BOTRYOGLOSSUM GROWTH

at 20 C. A difference of roughly 29 percent wet weight between the two treatments was measured at experimental day 28. FIGURE 3.10.5-4 shows a plot of wet weights ($\log_{10}$ transformed) through time for the two treatments. The difference in slopes between the two lines suggests a depressed rate of wet weight increase for the 20 C treatment when compared with the 16 C treatment.

It is important here to discuss differences observed in characteristics that could not be quantified between plants of the two treatments. Plants maintained at 16 C were well pigmented, turgid, and remained fairly free of epiphytes throughout the experimental period. At 20 C, plants were less well pigmented, quite flaccid, and heavily epiphytized by 28 days' growth.

Although epiphyte removal was attempted before wet weight measurements were made, this was not entirely possible; at 21 and 28 days, and for the 20 C treatment especially, wet weight values may therefore have been slightly inflated by the presence of epiphytes. The most luxurient and tenacious epiphyte among the treatments was an unidentified species of the Ectocarpales.

3.10.5.3 DISCUSSION

Investigations pertaining to the growth and physiology of Botryoglossum are lacking in the scientific literature; and it was not possible in this experiment to determine the optimum light intensity for growth or concentrations at which dissolved nutrients become limiting for this species. Slight between-treatment variation was measured in light intensity and nutrient concentration but these are assumed to be of insufficient magnitude to warrant exclusion of temperature as the principal variable promoting differences in growth rate among experimental treatments.

The results of this experiment indicate that the optimum temperature for growth of central California - collected specimens of Botryoglossum farlowianum, under laboratory conditions specified above, is approximately 16 C (61 F). Experimental temperatures of 14 C (57 F) and below, and 18 C (64 F) and above, contribute to depressed rates of growth when compared with 16 C (61 F). Along

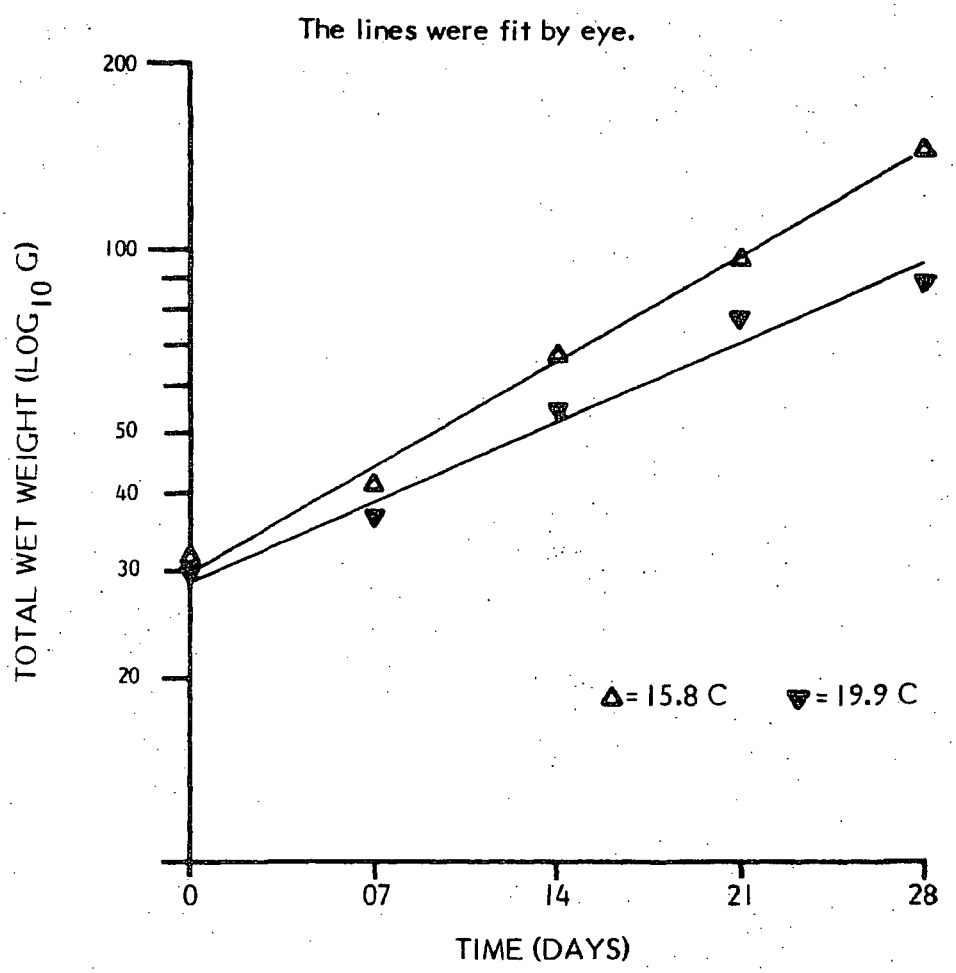


FIGURE 3.10.5-4

LOG-TRANSFORMED WET WEIGHT VERSUS TIME FOR RUN 25B: BOTRYOGLOSSUM GROWTH

with suboptimal growth rates reduced pigmentation and turgor were accompanied by enhanced growth of epiphytes at 20 C (68 F).

It is possible that 20 C (68 F) may represent a lethal temperature over the long run, but mortality was not evident at this temperature at the end of this 28 day experiment. A seawater temperature of 23.8 C was shown to elicit a lethal response from B. farlowianum after 96 hours exposure (see SECTION 2.10.28). This particular response could have been magnified or accelerated by the thermal shock effects at the initiation of the experiment.

3.10.6 IRIDAEA CORDATA GAMETOPHYTE EARLY DEVELOPMENT

The results of an experiment to determine the minimum, optimum and maximum temperatures for early development of the red alga Iridaea cordata are presented in this section. This species is closely related to I. flaccida (iridescent seaweed). In the Diablo Canyon area, I. cordata is principally distributed in the subtidal region, whereas I. flaccida occurs principally in the intertidal region. The results of experiments on both species (see SECTION 3.9 for a report on I. flaccida) thus provide a basis for comparing the effects of the thermal plume on subtidal versus intertidal algae.

A detailed review of the taxonomy, ecology, life history and thermal biology of Iridaea cordata was presented by PGandE (1979b). To orient the reader, a brief summary of the pertinent aspects of I. cordata's biology are presented here.

In the Diablo Canyon area, I. cordata occurs from exposed low intertidal to shallow subtidal depths (Burge and Schultz 1973). Two varieties are recognized within the species, I. cordata var. cordata and I. cordata var. splendens (Abbott 1971), both of which are recorded for the Diablo Canyon area. For this experiment, the var. splendens form was used.

The life history of I. cordata is the same as for I. flaccida (see SECTION 3.9 of this report). Reproduction and recruitment are accomplished exclusively by spores in I. cordata. Field studies have shown that, once established, the basal crust of I. cordata is perennial and capable of producing new blades vegetatively in an annual cycle. The longevity of the basal crusts is unknown but is presumed to be at least several years. From Hansen's data (1977) it would appear that recruitment is low and that the annual cycle of blade growth results almost entirely from vegetative growth of established basal crusts. Space and time limitations, however, preclude laboratory observations on mature I. cordata crusts. The experiment described in this sections follows the early development and growth of tetraspores and was designed to determine the minimum, optimum and maximum temperatures for early development at several light intensities of the gametophytic generation of this species.

The study objective was to determine the minimum, optimum and maximum temperatures for early development and initial growth of *I. cordata* gametophytes. The purpose was to provide an estimation of the effects of the power plant thermal discharge on recruitment and initial development of *I. cordata* gametophytes in Diablo Cove.

3.10.6.1 METHODS

A summary description of laboratory methods employed in the investigations into minimum, optimum and maximum temperatures for early development of *I. cordata* gametophytes is presented below. For a more detailed description of the laboratory methods see APPENDIX B, Procedure 321, and protocol (see APPENDIX C, experiment 21c).

Tetrasporangial blades were collected from an area north of Diablo Cove on 15 February 1980. They were transported to the laboratory in plastic bags and held in ambient seawater prior to use for the experiment. After briefly scrubbing the plants in 2 percent bleach in seawater and rinsing in sterile seawater, spore release was induced by drying the plants in covered dishes for 10-15 min at 13 C and then immersing them in seawater. A suspension of spores was used to establish cultures on coverslips. After the spores had settled and attached (4 h) the cultures were placed on the algal gradient tables at the temperatures and light intensities specified in the experiment protocol (see APPENDIX C, experiment 21c).

Cultures were sampled after 1, 3, 6, 9, 12 and 15 days on the gradient tables and 30 sporelings per treatment were measured at each sample time as described in SECTION 3.9.1.2 except that no cell counts were made because of difficulty in obtaining accurate counts.

Size of the sporeling discs was measured as diameter (in μm), converted to area (μm^2), assuming that disc shape was a perfect circle as observed from above. Visual observations confirmed that sporeling discs nearly always approximated a circle in shape when normally developed.

3.10.6.2 RESULTS

A complete summary of the data and analytical results is presented in this section.

Temperature Data

A summary of the temperature data is presented in TABLE 3.10.6-1. The mean of the means calculated for the three light intensities at each target temperature is given as "mean of means" and this value is referred to in the following presentation of results.

Growth Through Time

Linear regression was used to fit a line to log transformed sporeling area versus time for each light intensity and temperature combination. FIGURES 3.10.6-1, 3.10.6-2 and 3.10.6-3 and TABLE 3.10.6-2 show the results of this analysis. The slopes of the regression lines indicate relative differences in the growth rate of the sporelings. At all light intensities, growth at 22.7 C was absent (negative slope) or negligible. By day 6, none of the sporelings contained over two cells and most had only partial pigmentation. These sporelings were still undeveloped by day 15 (see FIGURE 3.10.6-4). Sporelings at 19.8 C were also irregular in outline (see FIGURE 3.10.6-4).

A one-way analysis of variance was used to test for temperature effects on growth at each light intensity (excluding the 22.7 C treatments). The results, which are shown in TABLE 3.10.6-3, indicate a significant temperature effect at all light levels. Individual means were compared using the Student-Newman-Keuls Multiple Range test (SNK) and the results are shown in TABLE 3.10.6-4.

At 110 and 220 $\mu E/m^2/s$, optimum growth is in the range from 15.4-18.7 C. The decrease in growth at both colder (12.4-8.6 C) and warmer (19.8 C) temperatures is statistically significant. At 165 $\mu E/m^2/s$, peak growth is at 17.8 C, with significantly lower growth rates at the other temperatures.

TABLE 3.10.6-1

SUMMARY OF TEMPERATURE DATA:
IRIDAEA CORDATA GAMETOPHYTE EARLY DEVELOPMENT

Target Temperature (C)	Target Light Intensity ($\mu E/m^2/s$)	Actual Temperature				
		Mean (S.D.)		Maximum	Minimum	Mean of means
9	110	8.5	0.22	9.3	8.2	8.6
	165	8.8	0.35	9.9	8.3	
	220	8.6	0.27	9.4	8.2	
13	110	12.5	0.65	13.8	11.3	12.4
	165	12.4	0.64	13.6	11.2	
	220	12.4	0.69	13.8	11.2	
16	110	15.3	0.79	16.9	13.9	15.4
	165	15.5	0.80	17.1	14.1	
	220	15.4	0.80	16.9	13.9	
18	110	17.8	0.87	19.7	16.2	17.8
	165	17.8	0.89	19.7	16.2	
	220	17.8	0.91	19.6	16.2	
19	110	18.6	0.80	19.5	17.2	18.7
	165	18.7	0.80	19.9	17.3	
	220	18.7	0.83	19.7	17.2	
20	110	19.9	0.99	21.0	18.1	19.8
	165	19.7	1.00	20.8	17.9	
	220	19.7	1.05	21.1	17.9	
22	110	22.6	0.65	23.5	21.5	22.7
	165	22.7	0.68	23.9	21.5	
	220	22.7	0.71	23.5	21.4	

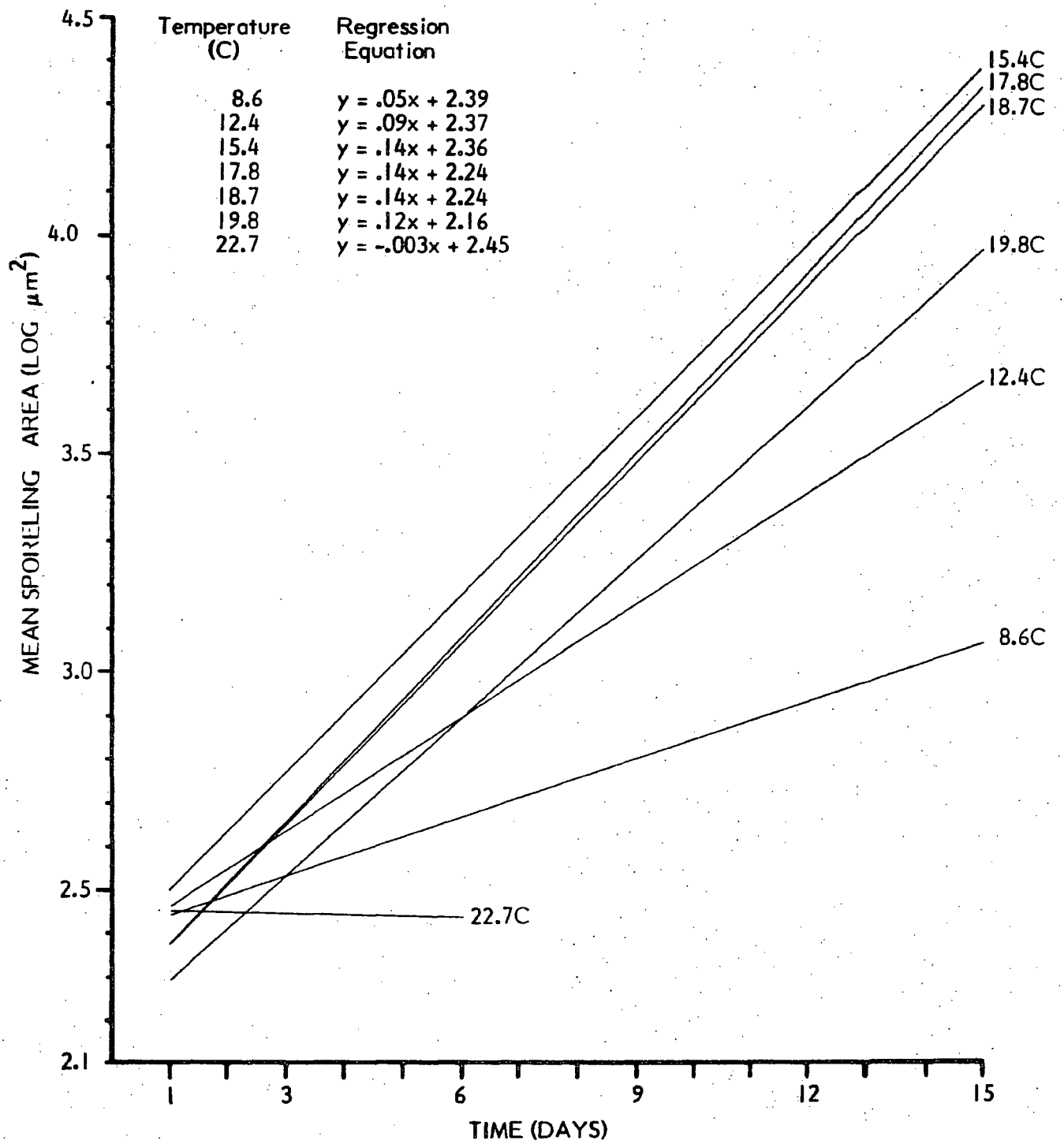


FIGURE 3.10.6-1

SPORELING DISC AREA VERSUS TIME AT 110 $\mu E/m^2/s$:
IRIDAEA CORDATA GAMETOPHYTE EARLY DEVELOPMENT

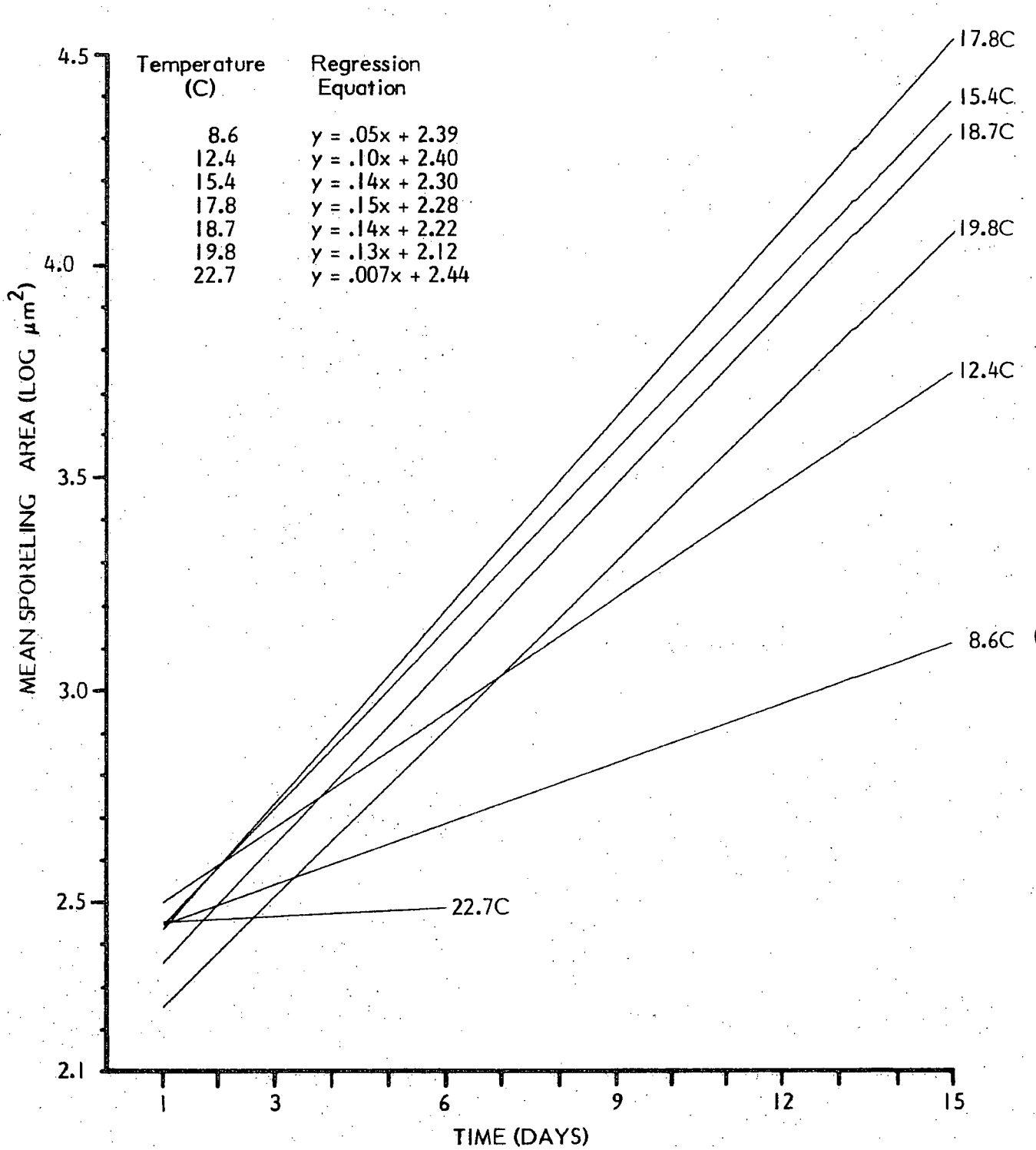


FIGURE 3.10.6-2

SPORELING DISC AREA VERSUS TIME AT 165 $\mu E/m^2/s$:
IRIDAEA CORDATA GAMETOPHYTE EARLY DEVELOPMENT

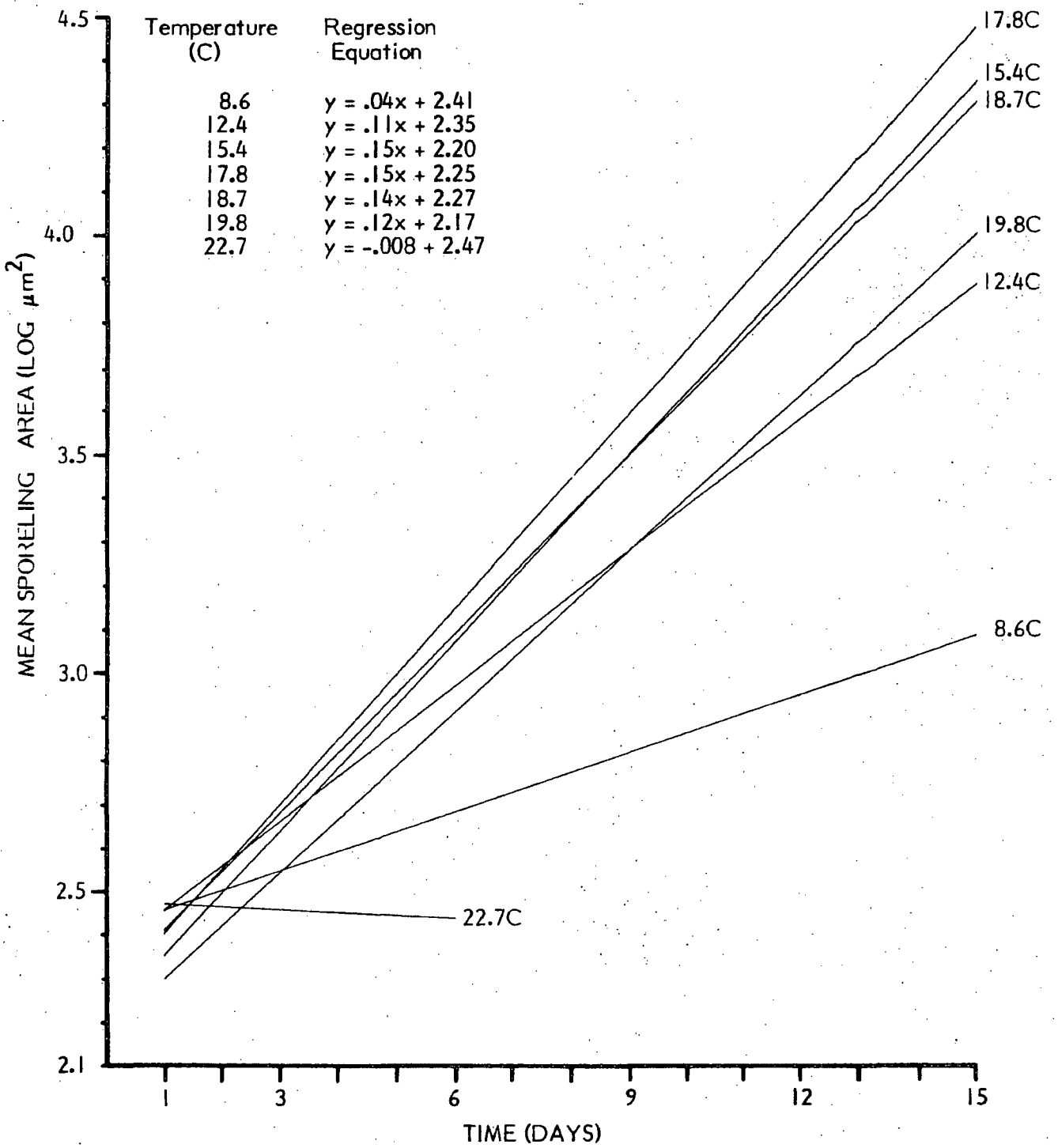


FIGURE 3.10.6-3

SPORELING DISC AREA VERSUS TIME AT 220 $\mu E/m^2/s$:
IRIDAEA CORDATA GAMETOPHYTE EARLY DEVELOPMENT

TABLE 3.10.6-2

LOG_{10} OF MEAN SPORELING DISC AREA (μm^2)
AT DAYS 1, 3, 6, 9, 12 AND 15:
IRIDAEA CORDATA GAMETOPHYTE
EARLY DEVELOPMENT

Cultures in which erect blade initiation was observed are indicated by "*".

Temperature (C)	Days					
	1	3	6	9	12	15
Light Intensity: 110 $\mu E/m^2/s$						
8.6	2.53	2.50	2.63	2.84	3.03	3.13
12.4	2.51	2.64	2.93	3.09	3.54	3.78
15.4	2.52	2.68	3.20	3.67	4.06	4.30*
17.8	2.46	2.48	3.19	3.55	3.98	4.33*
18.7	2.46	2.60	3.03	3.54	3.90	4.33
19.8	2.41	2.53	2.69	3.25	3.73	4.02
22.7	2.44	2.47	2.43	--	--	--
Light Intensity: 165 $\mu E/m^2/s$						
8.6	2.51	2.54	2.61	2.84	3.00	3.19
12.4	2.51	2.74	2.95	3.21	3.60	3.89
15.4	2.51	2.55	3.24	3.68	4.04	4.36*
17.8	2.51	2.54	3.28	3.79	4.13	4.53*
18.7	2.54	2.55	2.85	3.55	3.94	4.28*
19.8	2.46	2.49	2.60	3.28	3.79	4.09
22.7	2.45	2.47	2.48	--	--	--
Light Intensity: 220 $\mu E/m^2/s$						
8.6	2.52	2.50	2.65	2.80	3.00	3.09
12.4	2.53	2.64	2.95	3.25	3.63	3.99
15.4	2.44	2.47	3.18	3.68	4.11	4.41*
17.8	2.47	2.57	3.23	3.60	4.10	4.43*
18.7	2.51	2.55	3.08	3.68	4.07*	4.35*
19.8	2.43	2.48	2.67	3.33	3.69	4.04
22.7	2.47	2.47	2.45	--	--	--

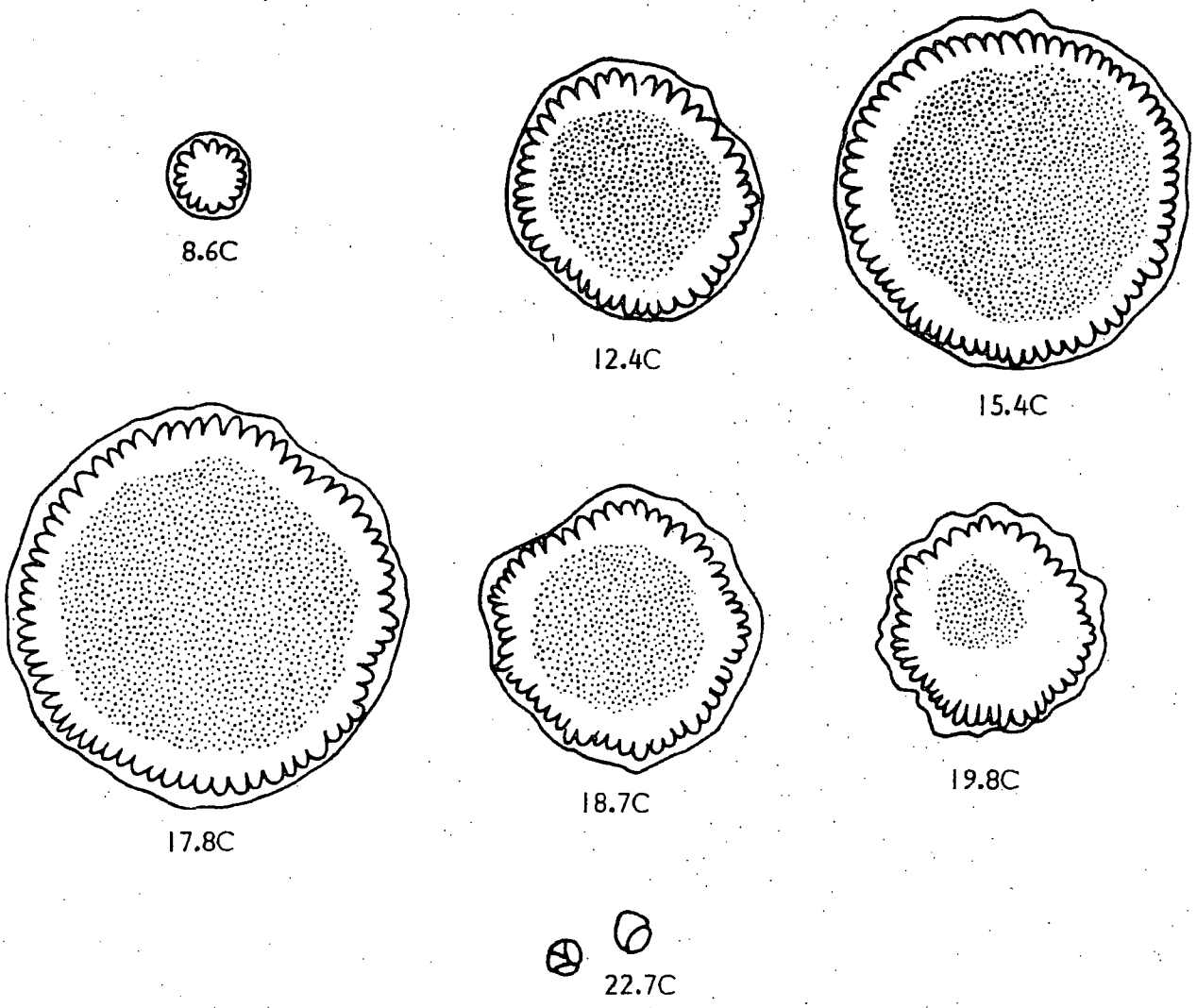


FIGURE 3.10.6-4

COMPARISON OF SPORELING DISC SIZE AT DAY 15, 220 $\mu E/m^2/s$:
IRIDAEA CORDATA GAMETOPHYTE EARLY DEVELOPMENT

Cells of peripheral colorless zone drawn
Central dark red area shaded.

TABLE 3.10.6-3

ONE-WAY ANALYSIS OF VARIANCE ON LOG_{10} SPORELING DISC AREA AT DAY 15: IRIDAEA CORDATA GAMETOPHYTES EARLY DEVELOPMENT

Significant values at p = 0.1 are indicated by "*".

Light Intensity ($\mu E/m^2/s$)	Source	Degrees of Freedom	Sums of Squares	Mean Squares	f_{CALC}
220	Temperature	5	38.920	7.784	435.8*
	Error	174	3.108	.018	
	Total	179	42.028		
165	Temperature	5	34.585	6.917	310.5*
	Error	174	3.877	.022	
	Total	179	38.462		
110	Temperature	5	33.864	6.773	260.8*
	Error	174	4.519	.026	
	Total	179	38.382		

TABLE 3.10.6-4

STUDENT-NEWMAN-KEULS (SNK) MULTIPLE RANGE TEST
ON LOG_{10} SPORELING DISC AREA AT DAY 15:
IRIDAEA CORDATA GAMETOPHYTE
EARLY DEVELOPMENT

Means connected by a common line are not statistically different (p = 0.01).

		K =				
		2	3	4	5	6
	Q 01	3.643	4.120	4.403	4.603	4.757
110	LSR ($S\bar{y}$ = .029)	.107	.121	.130	.136	.140
165	LSR ($S\bar{y}$ = .027)	.098	.111	.119	.124	.128
220	LSR ($S\bar{y}$ = .024)	.089	.101	.108	.113	.117

Light Intensity: 110 $\mu E/m^2/s$

Temperature (C) :	17.8	18.7	15.4	19.8	12.4	8.6
Log mean area (μm^2) :	4.32	4.31	4.29	3.99	3.77	3.11

Light Intensity: 165 $\mu E/m^2/s$

Temperature (C) :	17.8	15.4	18.7	19.8	12.4	8.6
Log mean area (μm^2) :	4.52	4.36	4.28	4.08	3.87	3.18

Light Intensity: 220 $\mu E/m^2/s$

Temperature (C) :	17.8	15.4	18.7	19.8	12.4	8.6
Log mean area (μm^2) :	4.43	4.41	4.34	4.02	3.98	3.08

Erect Blade Initiation

Initiation of erect blades was observed on day 12 only in the 18.7 C, 220 $\mu E/m^2/s$ treatment but by day 15, erect blades were observed in several treatments (see TABLE 3.10.6-2). A plot of blade initiation versus mean disc area and temperature (FIGURE 3.10.6-5) reveals that erect blades are initiated only when disc area reaches approximately 15,800 μm^2. There does not appear to be any direct temperature effect on blade initiation.

3.10.6.3 DISCUSSION

The optimum temperature range for early development of I. cordata gametophytes in culture is 15.4-18.7 C (59.7-65.7 F) when measured as total sporeling disc area after 15 days. At the intermediate light level tested (165 $\mu E/m^2/s$) disc area was statistically greater at 17.8 C (64.0 F) than at the next higher and lower temperatures. The greatest disc area was observed in this treatment, indicating that light saturation occurred at or near 165 $\mu E/m^2/s$. Absence of significant growth and loss of pigmentation at 22.7 C (72.9 F) indicate that this is a near lethal temperature for I. cordata under the laboratory conditions used.

There are no published accounts of sporeling development of I. cordata in the scientific literature comparable to the experiment reported here. However, Waaland (1973) studied growth of juvenile blades (2.5-3.0 mm long) of I. cordata from populations in Washington. In his experiment, optimum growth occurred at 10-14 C, 187-300 $\mu E/m^2/s$. The lower temperature for optimal growth obtained by Waaland compared to the results presented herein could be explained by the lower ambient temperatures to be found in higher latitudes. Other possible reasons for the differences are the age and generation of the experimental material used and possible genetic differences within the species (see discussion of varieties in PGandE 1979b).

Erect blade initiation occurred only in discs whose area reached or exceeded 15,850 μm^2 by day 15 and did not appear to be influenced directly by temperature.

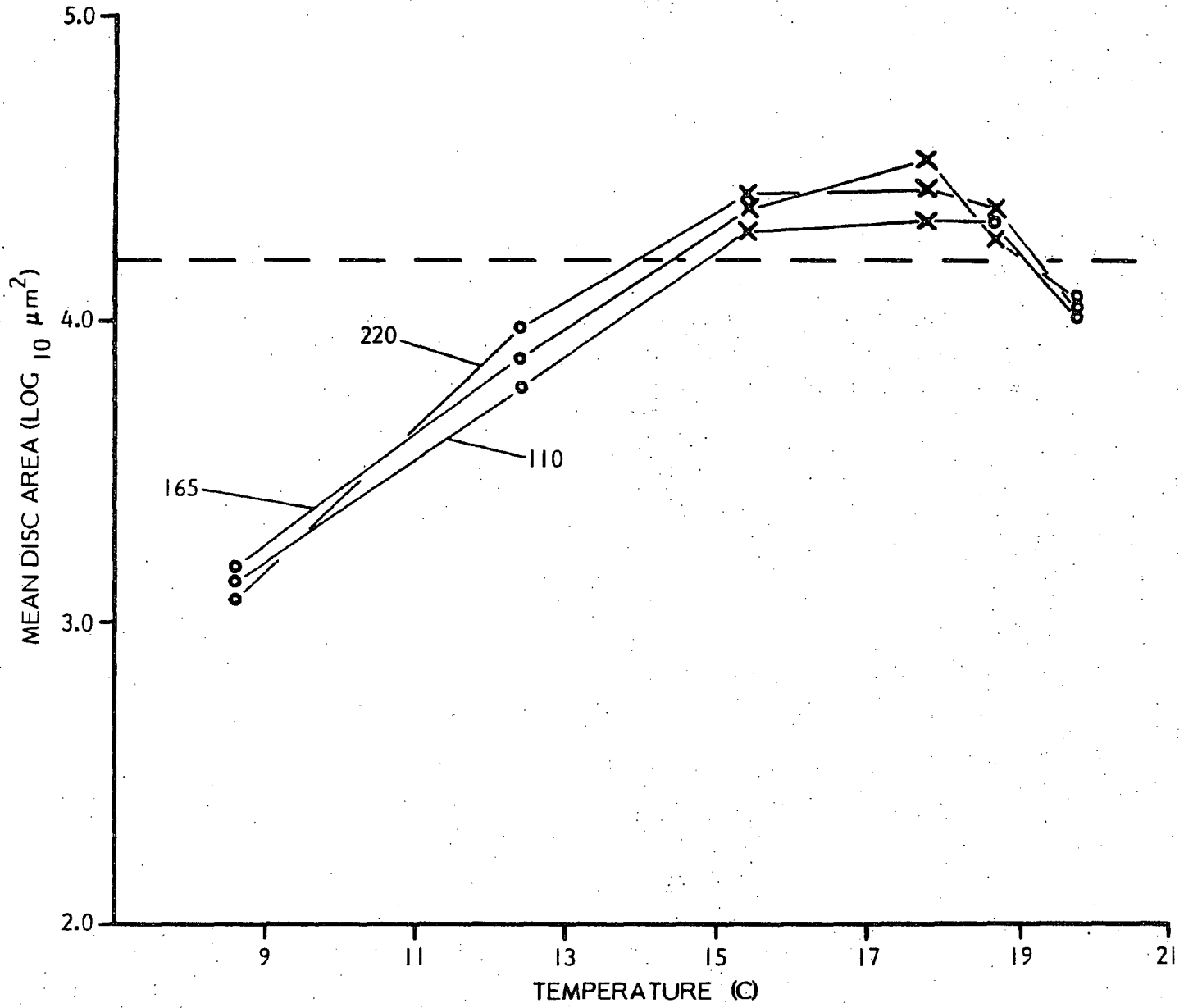


FIGURE 3.10.6-5

BLADE INITIATION RELATIVE TO SPORELING DISC AREA, TEMPERATURE AND LIGHT INTENSITY AT DAY 15: IRIDAEA CORDATA GAMETOPHYTE EARLY DEVELOPMENT

Light intensities shown as $\mu E/m^2/s$. "X" = blade initiation observed. "0" = blade initiation not observed. Horizontal dashed line at 4.2 $(\log_{10})\ \mu m^2$ level.

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

A series of behavioral experiments was designed to examine the thermal preference/avoidance response of selected fish and invertebrate species which are found in the Diablo Cove (discharge) area. This section contains our findings which were not previously reported for black-and-yellow/gopher rockfish (Sebastes chrysomelas/carnatus) (PGandE 1979). The following experiments with these rockfish include seven trials each of ten fish acclimated at 16 C and 12 C.

The experimental apparatus and methods employed in the temperature gradient experiments are briefly summarized below. For additional procedural details, the reader is referred to the laboratory procedure included in APPENDIX B (Procedure 306) and experimental protocols in APPENDIX C (experiments 6p, r, ac, m, n, s, t) of this report. The apparatus employed in these experiments has been described in detail in an early progress report (PGandE 1977, Section 6.2.2.6).

4.1.1 SPECIES DESCRIPTION

Adults of black-and-yellow/gopher rockfish are remarkably similar in appearance and habits. Two factors will usually enable the observer to separate the species:

- Color - although color pattern is essentially identical for both species, the gopher rockfish displays flesh tone dorsal splotches on a dark brown background whereas the black-and-yellow rockfish has yellow splotches on a black background.
- Bathymetric Distribution - the black-and-yellow rockfish inhabits waters shallower than approximately 15.3 m (50 ft) while the gopher rockfish, with some overlap by both species, is usually found below this level down to a depth of 55 m (180 ft).

The problem of differentiating between these two congeners dramatically increases in difficulty when dealing with juveniles of the size required for use in this experiment (approximately 30 mm standard length at time of capture). At

this stage both species can be found schooling together in shallow water with their distinctive color patterns not as yet developed. Transferring animals to the laboratory for further observation proved to be of limited value. As growth progressed, the juveniles took on the pattern of pigmentation found in the adults, but colors remained subdued, almost faded, preventing positive identification. This condition may be attributed to unnatural lighting and surroundings, plus a diet lacking in variety and, perhaps, the carotenoids or other constituents necessary for normal pigmentation. Whatever the cause, this problem necessitated lumping these animals together as black-and-yellow/gopher rockfish.

A technique that has been increasingly applied to species distinction problems such as encountered in Sebastes is biochemical genetics using the technique of electrophoresis. Electrophoresis can detect genetic differences between groups of fish. The technique is based on the naturally occurring distribution of genetic variants of proteins which allows a population or species to be characterized by its frequency of variants. Groups of individuals which share a common gene pool will have similar gene frequencies as determined by electrophoresis.

A study was conducted in 1979 to electrophoretically examine individuals identified as gopher and black-and-yellow rockfish from Morro Bay to determine to what extent genetic differences exist between two groups. Based on electrophoretic separation of the known adult samples, the study was to determine the species composition of a sample of juveniles who due to their small size could not be reliably identified as either gopher or black-and-yellow rockfish. The juvenile sample was collected from the laboratory test populations.

The classification procedure of the electrophoretic analysis (reported in PGandE 1981) was able to correctly classify approximately 70 percent of each adult species based on the biochemical genetic data alone. Based on these same criteria, 9 of the 20 juveniles (45 percent) were classified as gopher rockfish, while the remaining 11 were classified as black-and-yellow rockfish (55 percent). The biochemical genetic data can best be used to give probabilities of an individual's species membership, since no absolute differences exist between the two species. Based on these findings, it is assumed that the Sebastes spp.

specimens used in the following behavioral tests were comprised of 45 percent gopher rockfish and 55 percent black-and-yellow rockfish.

4.1.2 METHODS

A sample of juvenile rockfish was collected, using a 12 x 12 ft diver-operated lift net, from the area just outside the south breakwater of the Diablo Canyon Power Plant intake cove. All collections for this experiment were made in November and December 1978. The fish were immediately transported to the Thermal Effects Laboratory and held in several 4 x 8 x 2 ft fiberglass tanks at ambient seawater temperature. These fish were fed a maintenance diet of brine shrimp for an average period of approximately 90 days. During this period of time, 96-hour median lethal temperature tests and critical thermal maximum tests were performed on the laboratory population.

All of the collected fish were pooled and not kept separate by collection periods. Therefore some proportion of the fish (< 25 percent) were held in the laboratory for longer periods of time, but the majority of the pooled test specimens were held for less than 60 days before their use in the thermal preference experiments. The effect of holding these fish in the laboratory is unknown; however, some temperature preference change may have occurred due to nutrition and or slightly elevated ambient laboratory seawater temperatures. During the holding period (minimum of 48 hours) any injured fish or fish displaying abnormal behavior (schooling, feeding, swimming and equilibrium) were removed from the test populations.

Following the observational holding period, the selected test specimens were transferred to acclimation containers and the water temperatures raised or lowered to the specified acclimation temperatures (12 or 16 C), at the rate of 1 C per day. The fish were held at these acclimation temperatures until acclimation was achieved as measured by no further change in thermal resistance in a series of daily critical thermal maximum (CTM) tests, conducted according to Procedure 308. The results of the CTM tests were employed to

establish that acclimation was occurring, but full acclimation was probably achieved in the additional 1- to 2-week holding period at acclimation temperatures following the CTM tests and prior to the preference tests.

The acclimated fish were carefully transferred by bucket to the gradient test chamber upon completion of the gradient chamber setup described in APPENDIX B (Procedure 306). The pretest gradient temperature was established uniformly throughout the gradient at ±0.5 C of the respective 12 or 16 C acclimation temperatures. Photoperiod (lighting timer switch) was set to 14-hour light and 10-hour dark beginning with a lighted condition.

Positions of the fish in the gradient are recorded by three overhead 35 mm cameras with strobe lights that are automatically triggered by an electronic timer. The gradient temperature is measured electronically in each of the 25 gradient chamber compartments by thermistors imbedded in the chamber floor. The compartment temperatures are recorded manually on a YSI 81A recorder and automatically on an Autodata Nine temperature logger at 60-minute intervals. Digital time displays are positioned in the gradient chamber so that they appear in each photograph along with the compartment numbers.

The temperature range in the gradient is controlled by heating and chilling units with inflows to four heat exchangers, two at the ends and two on the bottom of the gradient chamber. Between runs, the hot and cold water inflows can be reversed to test any end preference effects. The gradient chamber is equipped with air supply airstones in each compartment to provide aeration and vertical circulation to destratify the gradient water temperatures.

The start of an experimental run is initiated by introducing the ten test fish (normally ten individuals depending upon size of organism and behavioral traits) into the gradient at their pre-experimental acclimation temperatures. The fish are left in this condition for a period of 15 to 17 hours. At the end of this time, the temperature controllers are set to -6 C acclimation temperature (ΔT) at the cold end, -3C ΔT, mid-gradient cold end, +3 C ΔT mid-gradient warm end, and +6 C ΔT warm end. The cameras and temperature recording systems are set to

5-minute intervals and operated at this interval for 60 minutes. At the end of this transition period, the recording rate is reset to 15-minute intervals. The gradient is then maintained for 120 minutes with no less than a 10 C difference between the hot and cold ends. After this stable gradient period, the gradients are routinely adjusted over the remaining course of the experiment in order to treat the chamber compartments with a range of temperatures and obviate compartment selections. This observation period continued for a period of not less than 7 hours and up to 24 hours. In this present experiment, the observation period from the establishment of the gradient was continued for a period of 8 hours (less than 24 hours from the introduction of the fish into the gradient chamber).

At the termination of the experiment, the fish were removed from the gradient chamber, anesthetized, weighed and measured and returned to the wild. The photographic films were developed and projected using three side-by-side projectors, and the compartment positions of the fish were recorded on data sheets for each photographic period. The recorded compartment temperatures at the time of each photograph were also entered on the data sheet and the data entered into the computer.

Mean selected temperatures (MST) for each recording interval were computed according to the formula:

$$MST = \frac{\sum X_i t_i}{\sum X_i}$$

where: MST = mean selected temperature, degrees C

X_i = number of fish in i^{th} compartment

t_i = temperature of ith compartment (degrees C)

i = 2 . . . 24 compartment with fish

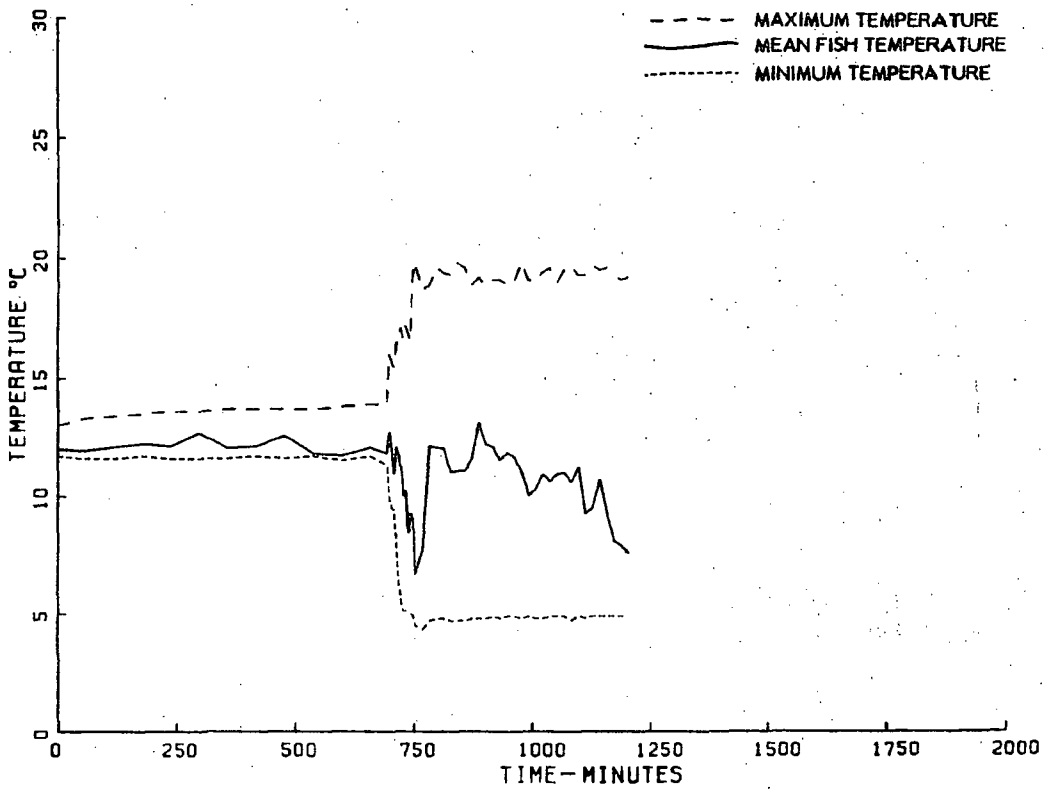
The fish and temperatures of the end compartments (compartments 1 and 25) were excluded from the calculation to obviate suspected positional effects on selection behavior.

4.1.3 RESULTS

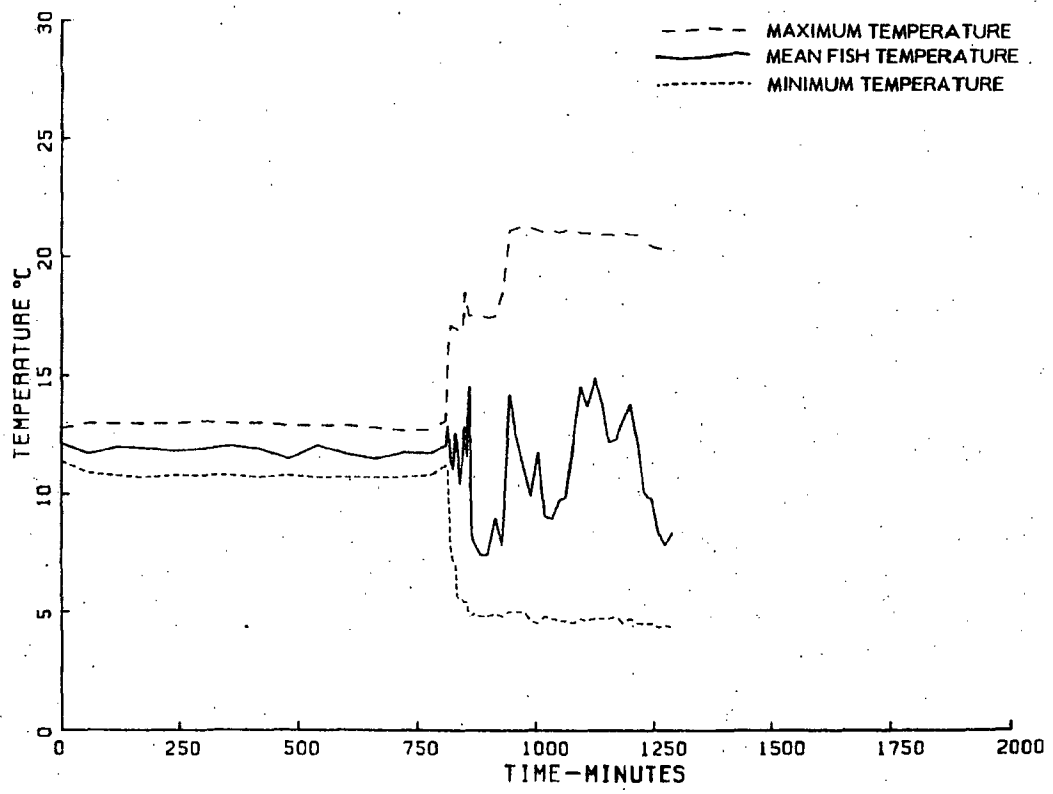
Results of the thermal gradient preference tests for blue rockfish acclimated at 12 and 16 C are expressed as the MST. The MST values for the 12 and 16 C acclimated fish were summarized by recording periods during the course of the experiment and are shown in FIGURES 4.1-1 and 4.1-2, respectively. The experiment was broken into an initial 10- to 16-hour period characterized by minimum thermal gradient. In practice a small gradient was present during this period due to the need to heat and chill in order to maintain a stable temperature (acclimation temperature). The largest temperature differences usually occurred in the last two or three warm end compartments. As seen in FIGURES 4.1-1 and 4.1-2, the MST for the 12 C and 16 C test groups during this acclimation period were very close to the nearly uniform gradient acclimation temperatures.

Following this 10- to 16-hour period of gradient chamber acclimation, an experimental temperature gradient was established by increasing the heating and cooling at opposite ends of the gradient. Observations were made at five minute intervals for 60 minutes during this transition to a wide range gradient. In most cases, the response of the 16 C acclimated fish to the introduced gradient was to move to cooler temperatures and remain in the cooler areas of the gradient throughout the experiment.

In the 16 C fish there was little or no increase in their MST over the course of the experiment, and the MST remained below 10 C to the end of the test. The behavioral response of the 12 C fish was dramatically different from the 16 C fish. The response of the 12 C fish to the thermal gradient was similar between tests (FIGURE 4.1-1, 6ad and 6aa). Both trial groups exhibited an immediate decrease in MST, a subsequent return to an MST nearly equal to their 12 C



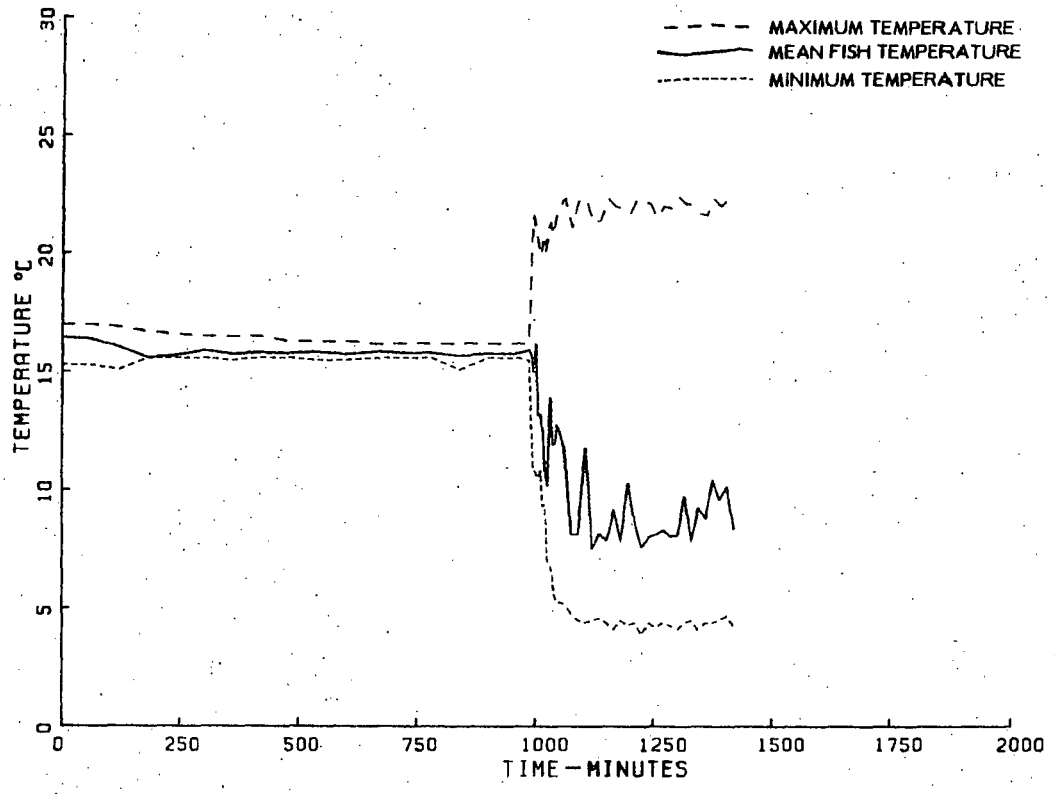
EXPERIMENT = 06S



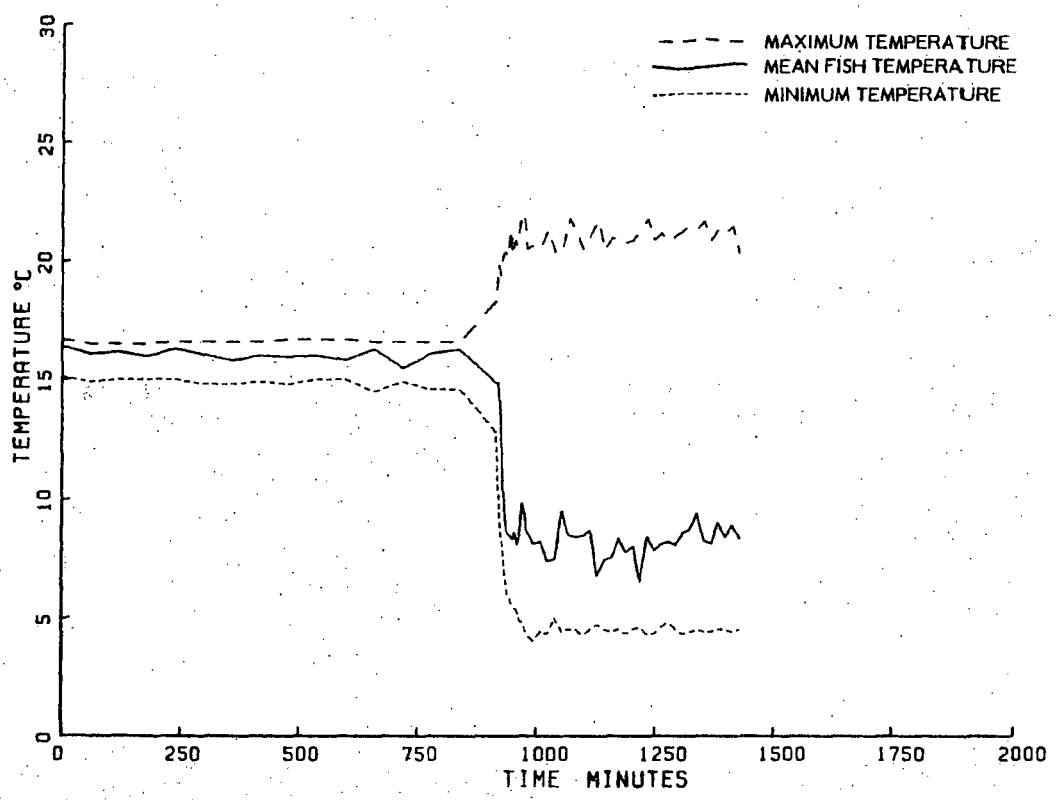
EXPERIMENT = 06N

FIGURE 4.1-1

THERMAL GRADIENT MEAN SELECTED TEMPERATURE VS. TIME OF JUVENILE BLACK AND YELLOW/GOPHER ROCKFISH (N=10) ACCLIMATED AT 12 C



EXPERIMENT = OGR



EXPERIMENT = OGP

FIGURE 4.1-2

THERMAL GRADIENT MEAN SELECTED TEMPERATURE VS. TIME OF JUVENILE BLACK AND YELLOW/GOPHER ROCKFISH (N=10) ACCLIMATED AT 16 C

acclimation temperature, and gradual decline in MST to the end of the experiment.

The calculated overall MSTs for the 12 C and 16 C acclimated fish were 11.7 C and 8.2 C, respectively. The MSTs for each experiment are shown in TABLE 4.1-1 along with the standard deviation and number of fish per test.

The frequency of occurrence of the 12 and 16 C acclimated fish with respect to temperature is illustrated in FIGURE 4.1-3 for experiments 6m, n, ac, p, r and s. The temperature gradient patterns of fish distribution varied considerably among experiments; however, the previously discussed patterns were repeated. The 16 C fish were most frequently distributed in the coolest portion of the gradient and the 12 C fish were distributed nearer their acclimation temperature. Examining all of the 1979 test results in a summary fashion, it can be stated that the 12 C fish preferred temperatures below 15 C and seemed to avoid temperatures above 17 C. The 16 C fish preferred temperatures below 10 C and commonly avoided temperatures above 12 C.

4.1.4 DISCUSSION

These results of the laboratory temperature gradient behavior tests will enable environmental assessments of the thermal discharge plume on the distribution of animal species in Diablo Cove. Many fish and some invertebrate species have been shown to respond significantly in their habitat selection to water temperatures. In this respect, there is a potential for some species to be thermally excluded from the discharge region (loss of habitat) or attracted to the discharge region.

In addition, these results of the temprature gradient behavior experiments were undertaken to gain information on the species' optimum temperature for growth. Based on experiments with freshwater fish species, it has been shown that there are examples of a direct relationship between preferred temperature and optimum temperature for growth.

TABLE 4.1-1

MEAN SELECTED TEMPERATURES (MST) OF BLACK-AND-YELLOW/GOPHER ROCKFISH IN THERMAL GRADIENT EXPERIMENTS

Experiment Code Number	Acclimation Temperature (°C)	MST (°C)	Standard Deviation	Number of Observations
6ac	16	9.2	4.2	541
06m	12	11.5	5.3	243
06n	12	12.1	4.9	230
06p	16	7.6	3.4	342
06r	16	7.8	4.3	217
06s	12	11.7	4.3	289
06t	12	11.4	4.0	370

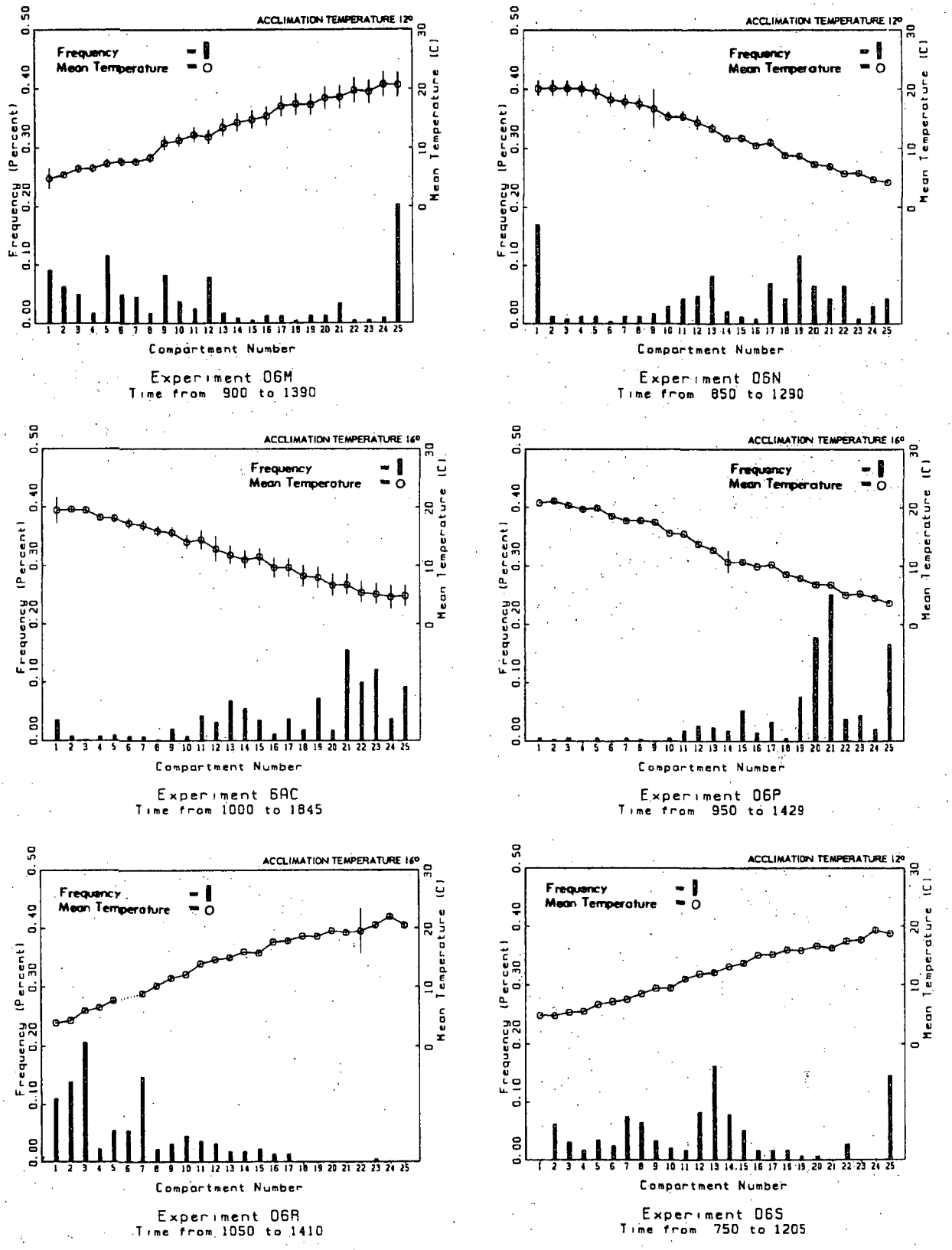


FIGURE 4.1-3

MEAN TEMPERATURE AND PERCENT FREQUENCY DURING OBSERVATION PERIOD (MINUTES) OF JUVENILE BLACK AND YELLOW/GOPHER ROCKFISH BY THERMAL GRADIENT COMPARTMENT

The results of the black-and-yellow/gopher rockfish temperature gradient behavior experiments have indicated a region of upper temperature avoidance. When integrated with measured or predicted thermal plume characteristics, an accounting of excluded habitat could possibly be estimated for a variety of power plant operating conditions.

The use of temperature preference results to estimate the optimum temperature for growth does not seem promising based on the species studied herein. From our findings we would predict that the optimum temperature for black-and-yellow/gopher rockfish growth would be 11.7 C (53.1 F) for 12 C (54 F) acclimated fish and 8.2 C (46.8 F) for 16 C (61 F) fish. The laboratory growth data indicates that the optimum temperature for growth is at or below 12 C (54 F) for this mixed species population. However, the agreement with the MST results may be a better finding than the 16 C (61 F) fish results since there seems to be an uncertainty about the effects of 16 C (61 F) acclimation.

4.2 BLUE ROCKFISH (SEBASTES MYSTINUS)

A series of behavioral experiments was designed to examine the thermal preference/avoidance response of selected fish and invertebrate species which are found in the Diablo Cove (discharge) area. This section contains our findings which were not previously reported for blue rockfish (Sebastes mystinus) (PGandE 1979). The following experiments with blue rockfish include eleven trials each of ten fish acclimated at 16 C and 12 C.

The experimental apparatus and methods employed in the temperature gradient experiments are briefly summarized below. For additional procedural details, the reader is referred to the laboratory procedure included in APPENDIX B (Procedure 306) and experimental protocols in APPENDIX C (experiments 6a, j, k, m, n, o, q, u, v) of this report. The apparatus employed in these experiments has been described in detail in an early progress report (PGandE 1977, Section 6.2.2.6).

4.2.1 SPECIES DESCRIPTION

The blue rockfish is one of 58 species in the genus reported from California. They have been reported from the Bering Sea to Punta Banda, Mexico (Miller and Lea 1972, addendum 1976).

Blue rockfish are non-migratory and concentrate in dense shoals in and near rocky pinnacle areas from the surface to depths of 90 m. Tagging studies have shown little movement between release locations. One extreme example is that in one case, a fish tagged and returned at Cape San Martin was recaptured 1,130 days later at the same place where it was released (Miller and Geibel 1973). During this three year study, over 1,600 fish were tagged and all returns were within 16.1 km of the release point. Of 168 returned fish, 142 traveled less than 1 mile, 23 traveled 1 to 6 miles and three moved 7, 12 and 15 miles. Similar non-migratory behavior prevailed in this and other study areas, indicating that adult blue rockfish remain in the area they settled into as juveniles.

A comprehensive life history study of the blue rockfish was accomplished by Miller, Odemar and Gotshall (1967). Eggs of blue rockfish are fertilized internally by sperm which is stored in the ovary. Copulation, though never observed, probably occurs shortly before ovulation. Yolk-sac (eyed) larvae develop within the egg capsule in the lumen of the ovary. Larval incubation is estimated to take 30 days. Spawning probably takes place once a year, and it is estimated that from 50,000 to 300,000 eggs per year are spawned, depending on the size of the individual and assuming only one spawning period per year. Blue rockfish larvae are about 3.5 mm TL at hatching, and are reported to occupy the epipelagic zone for several months (Miller and Geibel 1973). In the spring, juveniles 45-50 mm TL occupy the near shore kelp canopies. By fall, these juveniles reach a size of approximately 65-90 mm TL. Miller and Geibel (1973) summarized the central California sportfisheries, life history, catch analysis, behavioral and environmental relationships of the blue rockfish. The status of the rockfish resource and its management was documented by Miller and Hardwick (1973).

4.2.2 METHODS

A sample of juvenile blue rockfish were collected, using a 12 x 12 ft diver-operated lift net, from the area just outside the south breakwater of the Diablo Canyon Power Plant intake cove. All collections for this experiment were made in December 1978 and January 1979. The fish were immediately transported to the Thermal Effects Laboratory and held in several 4 x 8 x 2 ft fiberglass tanks at ambient seawater temperature. The fish were held in ambient (12 C) running seawater tanks and fed a maintenance diet of brine shrimp for a period of time no longer than 90 days. During this period of time 96-hour median lethal temperature tests and critical thermal maximum tests were performed on the population.

All of the collected fish were pooled and not kept separate by collection periods. Therefore some proportion of the fish (< 25 percent) were held in the laboratory for longer periods of time, but the majority of the pooled test specimens were held for 90 days before their use in the thermal preference experiments. The effect of holding these fish in the laboratory is unknown; however, some

temperature preference change may have occurred due to nutrition and or slightly elevated ambient laboratory seawater temperatures. During the holding period (minimum of 48 hours) any injured fish or fish displaying abnormal behavior (schooling, feeding, swimming and equilibrium) were removed from the test populations.

Following the observational holding period, the selected test specimens were transferred to acclimation containers and the water temperatures raised or lowered to the specified acclimation temperatures (12 or 16 C), at the rate of 1 C per day. The fish were held at these acclimation temperatures until acclimation was achieved as measured by no further change in thermal resistance in a series of daily critical thermal maximum (CTM) tests, according to procedure 308. The results of the CTM tests were employed to establish that acclimation was occurring, but full acclimation was probably achieved in the additional 1- to 2-week holding period at acclimation temperatures following the CTM tests and prior to the preference tests.

The acclimated fish were carefully transferred by bucket to the gradient test chamber upon completion of the gradient chamber setup described in APPENDIX B (Procedure 306). The pretest gradient temperature was established uniformly throughout the gradient at ±0.5 C of the respective 12 or 16 C acclimation temperatures. Photoperiod (lighting timer switch) was set to 14-hour light and 10-hour dark beginning with a lighted condition.

Positions of the fish in the gradient are recorded by three overhead 35 mm cameras with strobe lights that are automatically triggered by an electronic timer. The gradient temperature is measured electronically in each of the 25 gradient chamber compartments by thermistors imbedded in the chamber floor. The compartment temperatures are recorded manually on a YSI 81A recorder and automatically on an Autodata Nine temperature logger at 60-minute intervals. Digital time displays are positioned in the gradient chamber so that they appear in each photograph along with the compartment numbers.

The temperature range in the gradient is controlled by heating and chilling units with inflows to four heat exchangers, two at the ends and two on the bottom of the gradient chamber. Between runs, the hot and cold water inflows can be reversed to test any end preference effects. The gradient chamber is equipped with air supply airstones in each compartment to provide aeration and vertical circulation to destratify the gradient water temperatures.

The start of an experimental run is initiated by introducing the ten test fish (normally ten individuals depending upon size of organism and behavioral traits) into the gradient at their pre-experimental acclimation temperatures. The fish are left in this condition for a period of 15 to 17 hours. At the end of this time, the temperature controllers are set to -6 C acclimation temperature (ΔT) at the cold end, -3C ΔT, mid-gradient cold end, +3 C ΔT mid-gradient warm end, and +6 C ΔT warm end. The cameras and temperature recording systems are set to 5-minute intervals and operated at this interval for 60 minutes. At the end of this transition period, the recording rate is reset to 15-minute intervals. The gradient is then maintained for 120 minutes with no less than a 10 C difference between the hot and cold ends. After this stable gradient period, the gradients are routinely adjusted over the remaining course of the experiment in order to treat the chamber compartments with a range of temperatures and obviate compartment selections. This observation period continued for a period of not less than 7 hours and up to 24 hours. In this present experiment, the observation period from the establishment of the gradient was continued for a period of 8 hours (less than 24 hours from the introduction of the fish into the gradient chamber).

At the termination of the experiment, the fish were removed from the gradient chamber, anesthetized, weighed and measured and returned to the wild. The photographic films were developed and projected using three side-by-side projectors, and the compartment positions of the fish were recorded on data sheets for each photographic period. The recorded compartment temperatures at the time of each photograph were also entered on the data sheet and the data entered into the computer.

Mean selected temperatures (MST) for each recording interval were computed according to the formula:

$$MST = \frac{\sum X_i t_i}{\sum X_i}$$

where: MST = mean selected temperature, degrees C

X_i = number of fish in i^{th} compartment

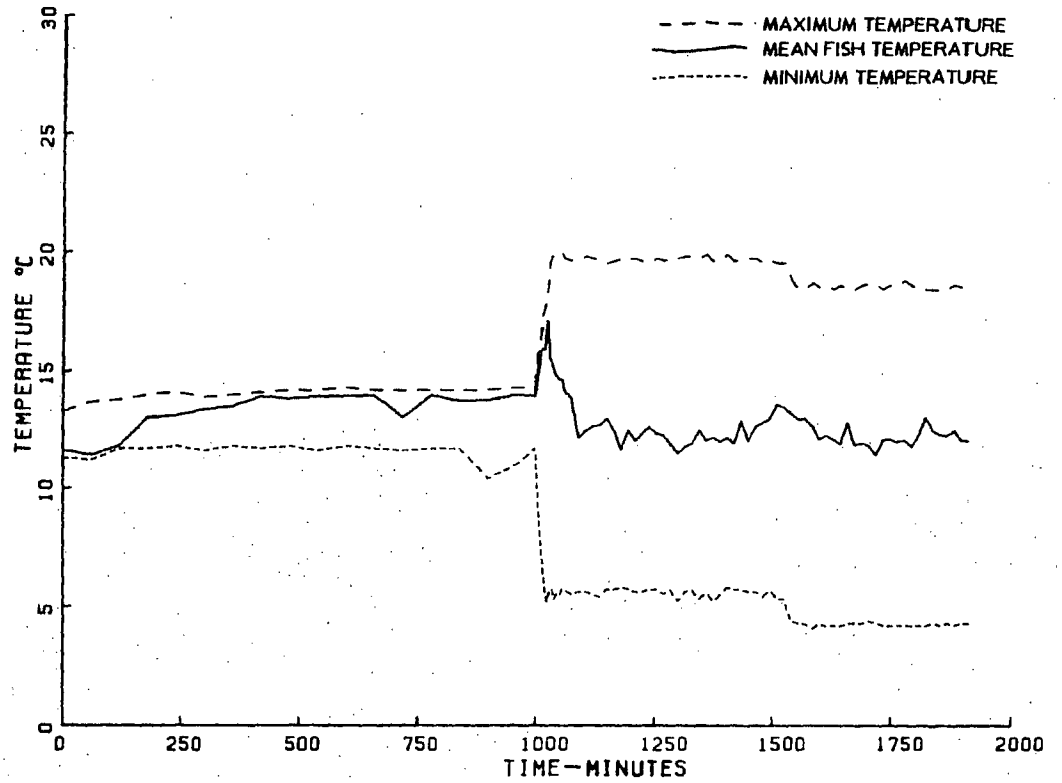
t_i = temperature of ith compartment (degrees C)

i = 2 . . . 24 compartment with fish

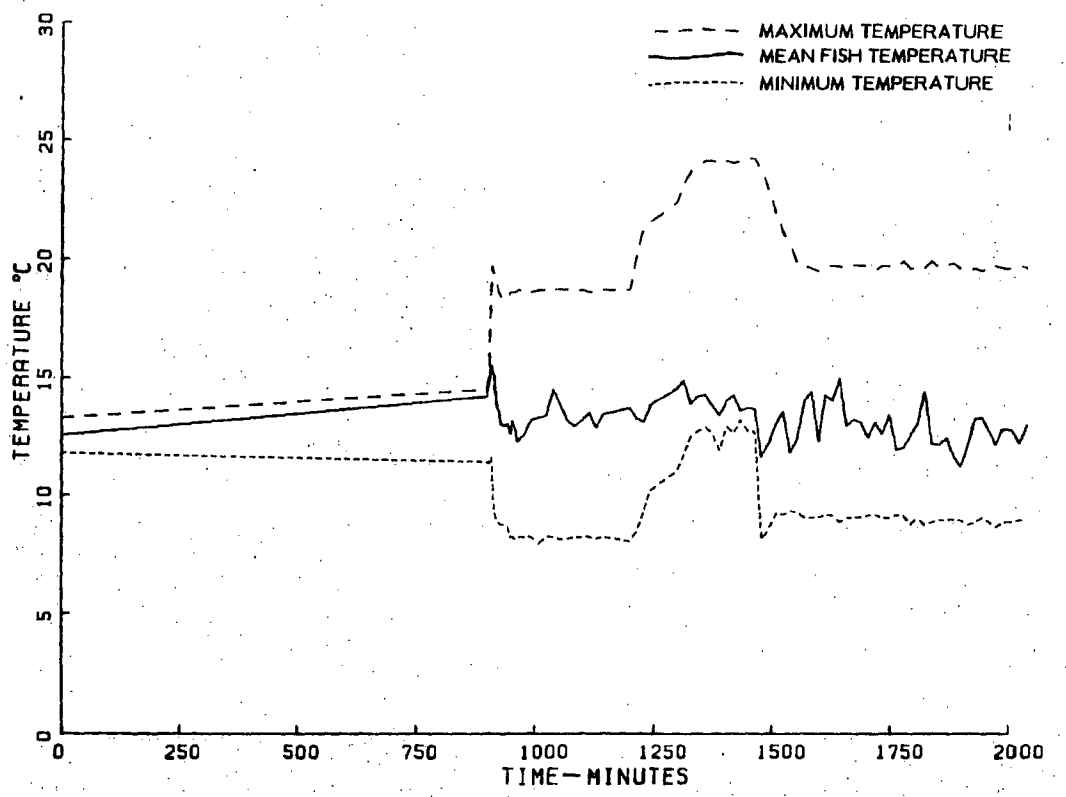
The fish and temperatures of the end compartments (compartments 1 and 25) were excluded from the calculation to obviate suspected positional effects on selection behavior.

4.2.3 RESULTS

Results of the thermal gradient preference tests for blue rockfish acclimated at 12 and 16 C are expressed as the MST. The MST values for the 12 and 16 C acclimated fish were summarized by recording periods during the course of the experiment and are shown in FIGURES 4.2-1 and 4.2-2, respectively. The experiment was broken into an initial 14- to 15-hour period characterized by minimum thermal gradient. In practice a small gradient was present during this period due to the need to heat and chill in order to maintain a stable temperature (acclimation temperature). The largest temperature differences usually occurred in the last two or three warm end compartments. As seen in FIGURE 4.2-2, the MST for the 16 C test groups during this acclimation period were very close to the nearly uniform gradient acclimation temperatures. The 12 C acclimated fish exhibited a slight increase in their preferred temperature during this habituation period.



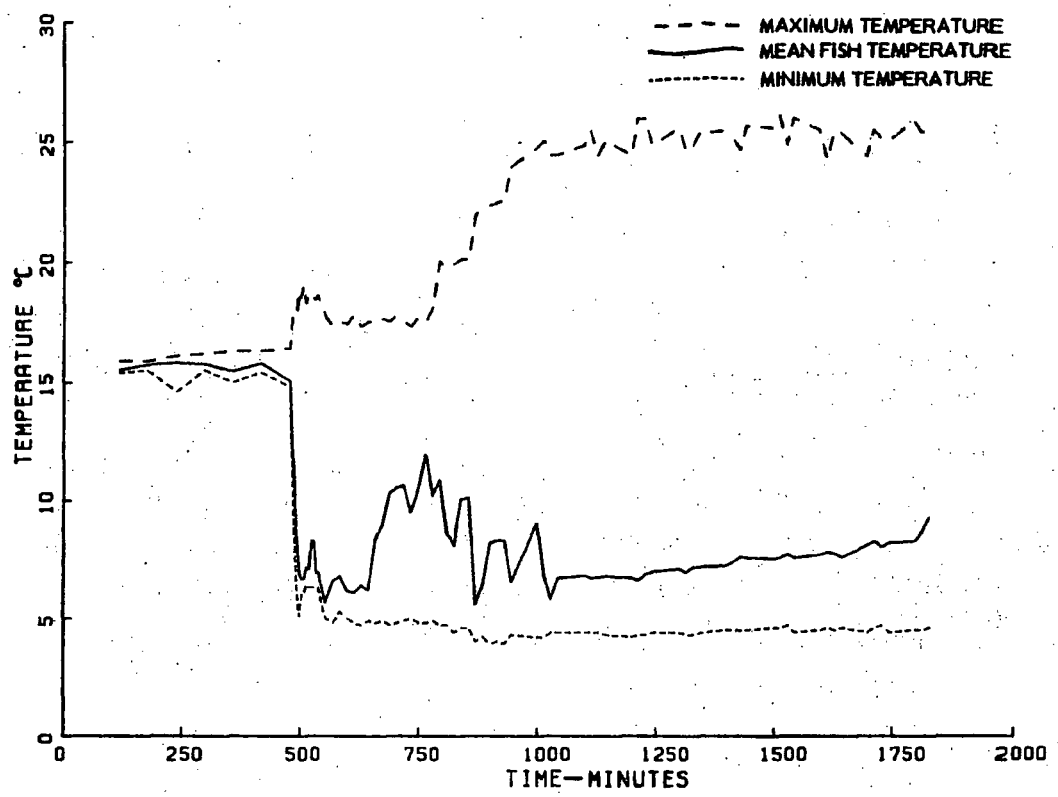
EXPERIMENT = 6AD



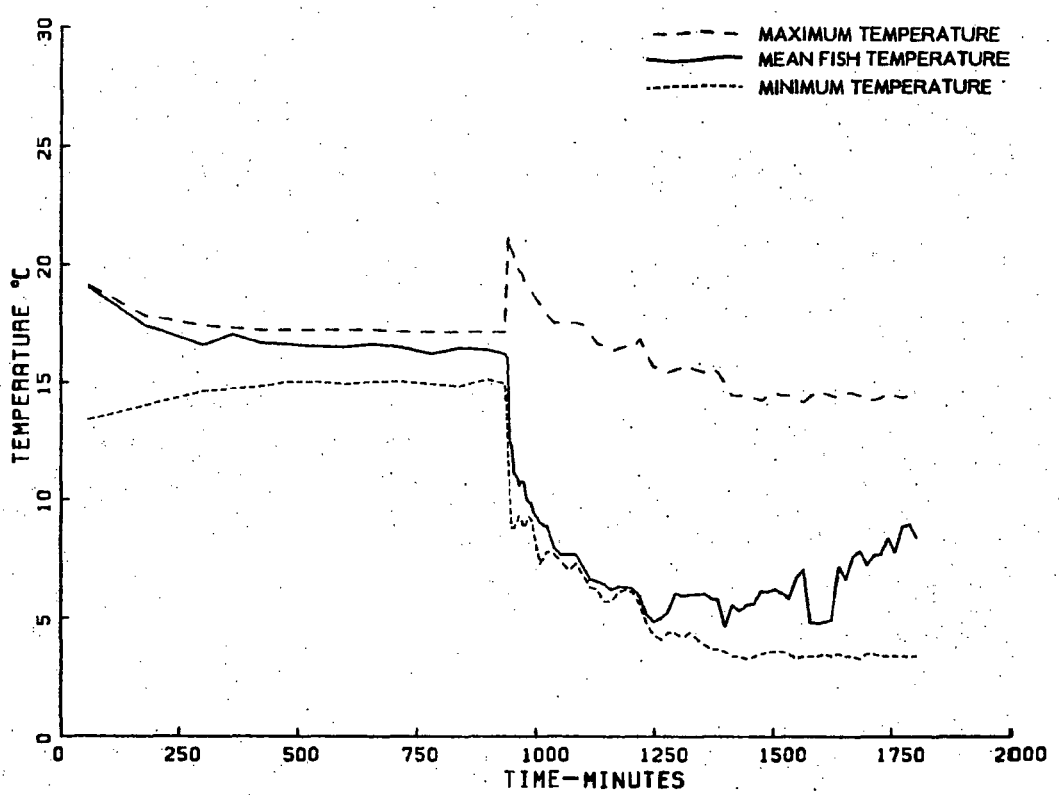
EXPERIMENT = 6AA

FIGURE 4.2-1

THERMAL GRADIENT MEAN SELECTED TEMPERATURE VS. TIME OF JUVENILE BLUE ROCKFISH (N=10) ACCLIMATED AT 12 C



EXPERIMENT = 06Z



EXPERIMENT = 06Y

FIGURE 4.2-2

THERMAL GRADIENT MEAN SELECTED TEMPERATURE VS. TIME OF JUVENILE BLUE ROCKFISH (N=10) ACCLIMATED AT 16 C

Following this 14- to 15-hour period of gradient chamber acclimation, an experimental temperature gradient was established by increasing the heating and cooling at opposite ends of the gradient. Observations were made at five minute intervals for 60 minutes during this transition to a wide range gradient. In most cases, the response of the 16 C acclimated fish to the introduced gradient was to move to cooler temperatures and remained in the cooler areas of the gradient throughout the experiment.

In the 16 C fish there was a gradual increase in their MST over the course of the experiment, but the MST remained below 10 C to the end of the test. The behavioral response of the 12 C fish was dramatically different from the 16 C fish. The response of the 12 C fish to the thermal gradient was notably similar between tests (FIGURE 4.2-1, 6ad and 6aa). Both trial groups exhibited an immediate increase in MST and a rapid return to an MST nearly equal to their 12 C acclimation temperature. Their MST remained very constant throughout the remaining 10 to 12 hours of gradient exposure.

The calculated overall MSTs for the 12 C and 16 C acclimated fish were 12.5 C and 9.3 C, respectively. The MSTs for each experiment are shown in TABLE 4.2-1 along with the standard deviation and number of fish per test.

The frequency of occurrence of 12 and 16 C acclimated fish with respect to temperature is illustrated in FIGURE 4.2-3 for experiments 6ag, 6aa, 6y, 6z, 6ad and 6w. The temperature gradient patterns of fish distribution varied considerably among experiments; however, the previously discussed patterns were repeated. The 16 C fish were most frequently distributed in the coolest portion of the gradient and the 12 C fish were distributed around their acclimation temperature. Examining all of the 1979 test results in a summary fashion, it can be stated that the 12 C fish preferred temperatures below 15 C (in most experiments they avoided temperatures above 17 C). The 16 C fish preferred temperatures below 12 C and commonly avoided temperatures above 10 C.

TABLE 4.2-1

MEAN SELECTED TEMPERATURE (MST)
FOR BLUE ROCKFISH THERMAL
GRADIENT EXPERIMENTS

Experiment	Acclimation Temperature (° C)	MST (°C)	Standard Deviation	Number of Observations
6aa	12	13.2	1.3	734
6ad	12	12.4	1.1	600
6ag	12	11.8	2.4	366
6af	16	15.6	1.1	328
6w	16	10.9	2.3	603
6y	16	7.3	3.0	622
6z	16	9.6	5.3	686

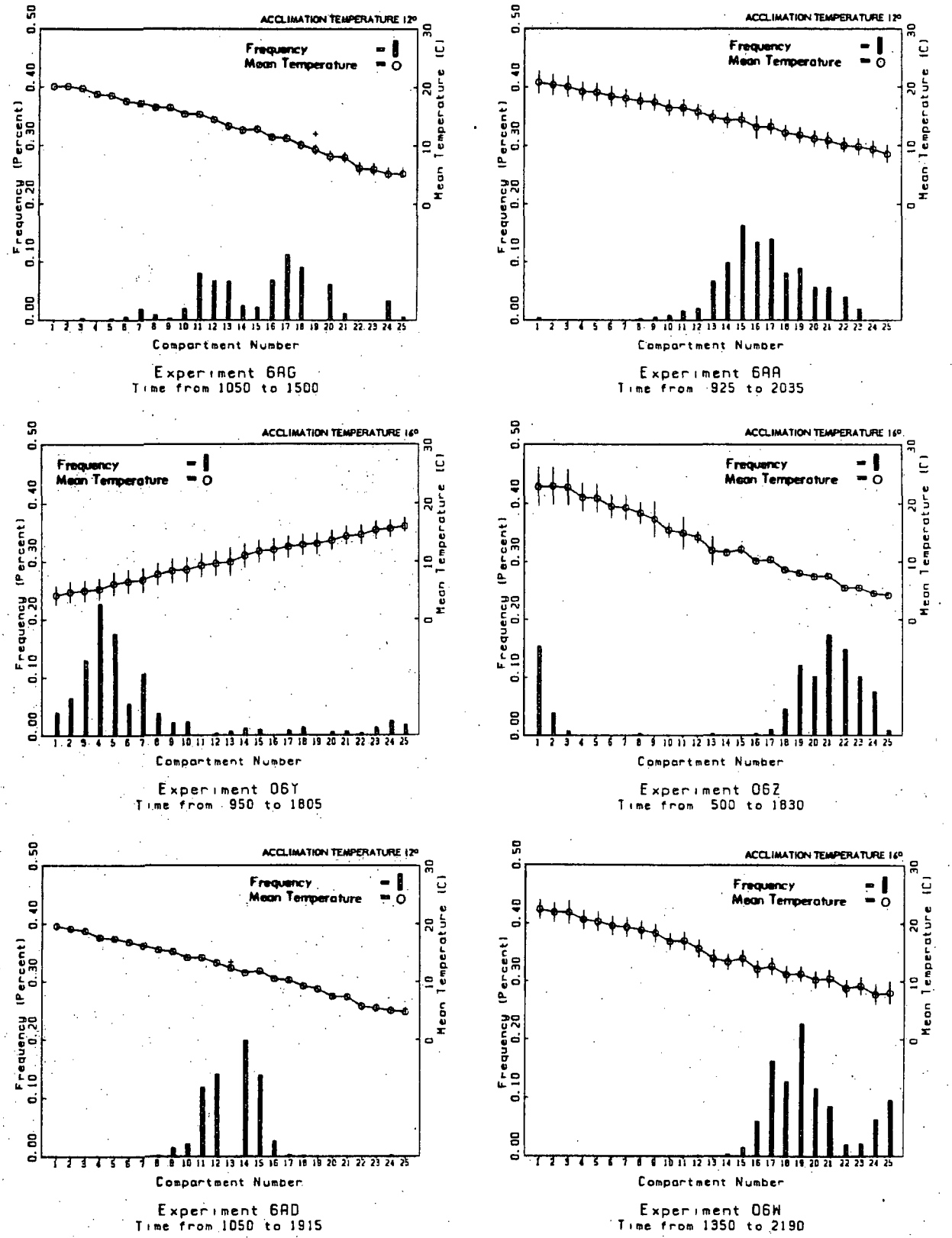


FIGURE 4.2-3

MEAN TEMPERATURE AND PERCENT FREQUENCY
DURING OBSERVATION PERIOD (MINUTES) OF
JUVENILE BLUE ROCKFISH
BY THERMAL GRADIENT COMPARTMENT

4.2.4 DISCUSSION

These results of the laboratory temperature gradient behavior tests will enable environmental assessments of the thermal discharge plume on the distribution of animal species in Diablo Cove. Many fish and some invertebrate species have been shown to respond significantly in their habitat selection to water temperatures. In this respect, there is a potential for some species to be thermally excluded from the discharge region (loss of habitat) or attracted to the discharge region.

In addition, these results of the temperature gradient behavior experiments were undertaken to gain information on the species' optimum temperature for growth. Based on experiments with freshwater fish species, it has been shown that there are examples of a direct relationship between preferred temperature and optimum temperature for growth. However, the relationship has been little researched in marine species.

Several authors have correlated the preferred temperature with habitat and life history characteristics. Medvick and Miller (1979) found the preferred temperature in the butterfly fish (Chaetodon) to correspond with that of its coral prey. They also found that juvenile damselfish and surgeonfish had higher preferred temperatures than the adults, and related this to the fact that these juveniles are commonly found in tide pools which are considerably warmer than the adult habitat. Terry and Stephens (1976) found the same relationship with adult and juvenile black surfperch (Embiotoca jacksoni). In the same study the dwarf surfperch, (Micrometrus minimus), was found to have a broad thermal preference. This was related to its habitat, which is consistently in the upper 1.5 m of the water column, and hence receives considerable seasonal temperature fluctuations. Other species of surfperch (Hypsurus caryi, Rhacochilus vacca, Phanerodon furcatus and Embiotoca jacksoni) were found to migrate into deeper water during the late summer and fall, and into shallower water during the winter and spring, to remain in their preferred temperatures.

Hill et al. (1975) related the effects of acclimation temperatures on the final preferred temperature to the habitat of the fish. They found that fish living in stream habitats that were subjected to considerable temporal fluctuations selected a certain temperature regardless of their initial acclimation temperature. On the other hand, acclimation had a strong effect on the preferred temperature of fish found in stable lake environments. Norris (1963) postulated that temperature selection in the opaleye, (Girella nigricans), functioned to limit the range of the species to temperatures that were not inhibitory to their reproduction. Roed (1979) found the preferred temperature of the stickleback, (Gasterosteus), to correlate with the temperature of its winter habitat, which is the minimum temperature it encounters. Brett (1971) found the preferred temperature of the sockeye salmon, (Oncorhynchus nerka), to correlate with the optimal temperature of many physiological processes. Among these were the growth rate, active metabolic rate, performance rate, and circulation rate.

The results of the blue rockfish temperature gradient behavior experiments have reasonably established a region of both upper and lower temperature avoidance depending upon the thermal history of the fish. When integrated with measured or predicted thermal plume characteristics, an accurate accounting of excluded habitat can be assessed for a variety of power plant operating conditions.

The use of blue rockfish temperature preference results to estimate the optimum temperature for growth does not seem promising. Based on our findings we would predict that the optimum temperature for growth would be 9.3 C (48.7 F) for 16 C (61 F) acclimated fish and 12.5 C (54.5 F) for 12 C (54 F) acclimated fish. From our laboratory findings on 60-day measured growth in blue rockfish (preceding SECTION 3.10.1), the optimum temperature for growth is between 12 and 16 C (54 and 61 F). Though the true optimum temperature for growth may be an intermediate temperature which was not tested between 16 and 18 C (61 and 64 F), the lack of correspondence between the preferred temperature and optimum growth temperature and the wide variance in the preferred temperature MST for the 16 C (61 F) acclimated fish would seem to preclude an accurate inference of optimum growth temperature from our preferred temperature findings. The correspondence for 12 C (54 F) fish was very close to the lower end of the optimum growth temperature range.

4.3 CABEZON (SCORPAENICHTHYS MARMORATUS)

A series of behavioral experiments were designed to examine the thermal preference/avoidance response of selected fish and invertebrate species which are found in the Diablo Cove (discharge) area. This section contains our findings which were not previously reported for cabezon (Scorpaenichthys marmoratus). The following experiments with cabezon include eleven trials of one fish per trial using fish acclimated at 16 C.

The experimental apparatus and methods employed in the temperature gradient experiments are briefly summarized below. For additional procedural details, the reader is referred to the laboratory procedure included in APPENDIX B (Procedure 306) and experimental protocols in APPENDIX C (experiments 6a, j, k, m, n, o, q, u, v) of this report. The apparatus employed in these experiments has been described in detail in an early progress report (PGandE 1977, Section 6.2.2.6).

4.3.1 SPECIES DESCRIPTION

The cabezon is one of the forty-two sculpins known to inhabit the marine waters off California. It has been reported from Sitka, Alaska, to Point Abreojos, Baja California, Mexico.

This species occurs from the intertidal, where it has been found in tidepools, to depths of 76 m (250 ft) (Fitch and Lavenberg 1971, Miller and Lea 1976, Roedel 1953, Feder et al. 1974, and Limbaugh 1955). More specifically, the habitat occupied depends on the stage of development. Eggs are laid in large masses, usually on cleared flat sloping rocks (Limbaugh 1955). The larvae are found in the plankton, most common near shore but also found as far as 200 miles offshore (O'Connel 1953). At a length of approximately 40 mm, they become demersal often occupying tide pools in large numbers. As they grow older and larger they migrate into deeper water.

The life history of cabezon involves external fertilization in the subtidal. The spawning season has been defined by O'Connel (1953) by examining the presence of larvae in the plankton. Spawning commences in late October and probably ends in March, with the peak occurring in January. Cabezon can spawn more than once per season. The eggs are laid commonly on flat sloping rocks. Nests are estimated to have 40,000 to 100,000 eggs. Cabezon may return to the same nesting site year after year according to Limbaugh (1955). The adults tenaciously guard their nest. After an incubation period of two full weeks the eggs hatch and the larvae are pelagic from October through April. Cabezon is an excellent food fish, sought after by sportfisherman and spearfisherman (Howorth 1977). Cabezon is not taken commercially.

4.3.2 METHODS

A sample of cabezon was collected from inside Morro Bay, California. The specimens were collected by trawl, placed in aerated tubs and transported to the Diablo laboratory. The fish were held in ambient (12 C) running seawater tanks and fed a maintenance diet of cut squid for an average period of approximately 60 days. During this period of time 96-hour median lethal temperature tests and critical thermal maximum tests were performed on the population.

All of the collected fish were pooled and not kept separate by collection periods. Therefore some portion of the fish (< 25 percent) was held in the laboratory longer than others, but the majority of the pooled test specimens were held for less than 60 days before their use in the thermal preference experiments. The effect of holding these fish in the laboratory is unknown; however, some temperature preference change may have occurred due to nutrition and ambient laboratory seawater temperatures which are lower than Morro Bay temperatures. During the holding period (minimum of 48 hours) any injured fish or fish displaying abnormal behavior (schooling, feeding, swimming and equilibrium) were removed from the test populations.

Following the observational holding period, the selected test specimens were transferred to acclimation containers and the water temperatures raised or lowered to the specified acclimation temperatures (16 C), at the rate of 1 C per day. The fish were held at these acclimation temperatures until acclimation was achieved as measured by no further change in thermal resistance in a series of daily critical thermal maximum (CTM) tests, according to Procedure 308. The results of the CTM tests were employed to establish that acclimation was occurring, but full acclimation was probably achieved in the additional 1- to 2-week holding period at acclimation temperatures following the CTM tests and prior to the preference tests.

The acclimated fish were carefully transferred by bucket to the gradient test chamber upon completion of the gradient chamber setup described in APPPENDIX B (Procedure 306). The pretest gradient temperature was established uniformly throughout the gradient at ±0.5 C of the 16 C acclimation temperatures. Photoperiod (lighting timer switch) was set to 14-hour light and 10-hour dark beginning with a lighted condition.

Positions of the fish in the gradient are recorded by three overhead 35 mm cameras with strobe lights that are automatically triggered by an electronic timer. The gradient temperature is measured electronically in each of the 25 gradient chamber compartments by thermistors imbedded in the chamber floor. The compartment temperatures are recorded manually on a YSI 81A recorder and automatically on an Autodata Nine temperature logger at 60-minute intervals. Digital time displays are positioned in the gradient chamber so that they appear in each photograph along with the compartment numbers.

The temperature range in the gradient is controlled by heating and chilling units with inflows to four heat exchangers, two at the ends and two on the bottom of the gradient chamber. Between runs, the hot and cold water inflows can be reversed to test any end preference effects. The gradient chamber is equipped with air supply airstones in each compartment to provide aeration and vertical circulation to destratify the gradient water temperatures.

The start of an experimental run is initiated by introducing the test fish (a single individual) into the gradient at their pre-experimental acclimation temperatures. In the case of the cabezon the acclimation temperature was always 16 C. The fish was left in this condition for a period of 15 to 17 hours. At the end of this time, the temperature controllers are set to -6 C acclimation temperature (ΔT) at the cold end, -3C ΔT, mid-gradient cold end, +3 C ΔT mid-gradient warm end, and +6 C ΔT warm end. The cameras and temperature recording systems are set to 5-minute intervals and operated at this interval for 60 minutes. At the end of this transition period, the recording rate is reset to 15-minute intervals. The gradient is then maintained for 120 minutes with no less than a 10 C difference between the hot and cold ends. After this stable gradient period, the gradients are routinely adjusted over the remaining course of the experiment in order to treat the chamber compartments with a range of temperatures and obviate compartment selections. This observation period continued for a period of not less than 7 hours and up to 24 hours. In this present experiment, the observation period from the establishment of the gradient was continued for a period of 5 hours (less than 24 hours from the introduction of the fish into the gradient chamber) and the gradient direction was reversed midway in the observation period.

At the termination of the experiment, the fish were removed from the gradient chamber, anesthetized, weighed and measured and returned to the wild. The photographic films were developed and projected using three side-by-side projectors, and the compartment positions of the fish were recorded on data sheets for each photographic period. The recorded compartment temperatures at the time of each photograph were also entered on the data sheet and the data entered into the computer.

Mean selected temperatures for each recording interval were computed according to the formula:

$$MST = \frac{X_i t_i}{X_i}$$

where: MST = mean selected temperature, degrees C

X_i = number of fish in i^{th} compartment

t_i = temperature of ith compartment (degrees C)

i = 2 . . . 24 compartment with fish

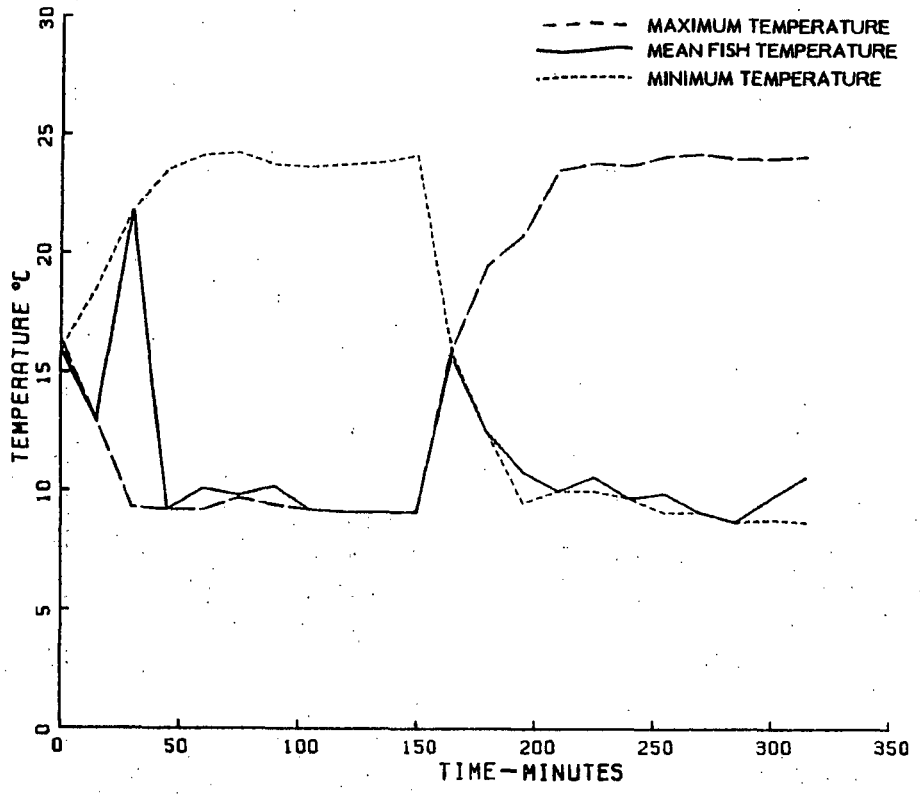
The fish and temperatures of the end compartments (compartments 1 and 25) were excluded from the calculation to obviate suspected positional effects on selection behavior. Overall MST values were also calculated separately for each half of the experiment (reversed gradient periods).

4.3.3 RESULTS

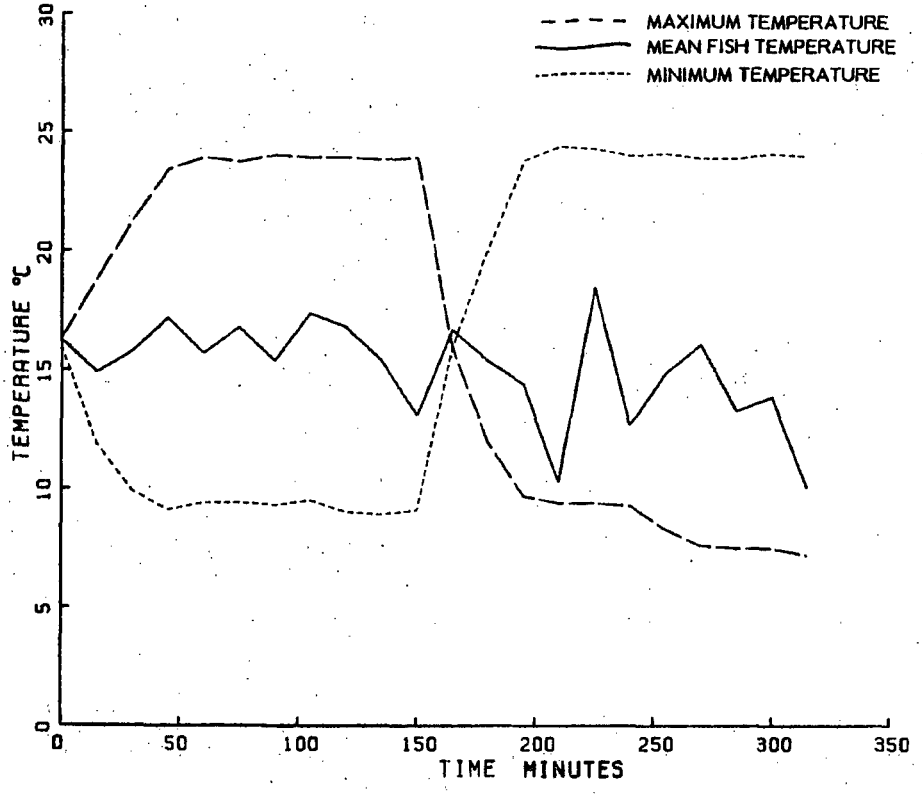
Results of the thermal gradient preference tests for cabezon acclimated at 16 C are expressed as the MST. The MST values for the 16 C acclimated fish were summarized by recording periods during the course of the experiment and are shown as two examples in FIGURE 4.3-1. The experiment was broken into an initial 6-hour period characterized by minimum thermal gradient. In practice a small gradient was present during this period due to the need to heat and chill in order to maintain a stable temperature (acclimation temperature).

Following this 6-hour period of gradient chamber acclimation, an experimental temperature gradient was established by increasing the heating and cooling at opposite ends of the gradient. Observations were made at five-minute intervals for 60 minutes during this transition to a wide range gradient. In approximately half the cases, the response of the fish to the introduced gradient was to move towards the cooler temperatures and remain there and in the other half seek their 16 C acclimation temperature. The MST values of the fish ranged widely from the hot to cold temperatures during the transition period.

The MST values were calculated for each experimental period (two periods per experiment, reversed gradient) and are summarized in TABLE 4.3-1. MST variance, shown as standard deviation in TABLE 4.3-1, was relatively uniform throughout the experiments; however, as noted above, the MST values are widely



EXPERIMENT = 6AR



EXPERIMENT = 6AS

FIGURE 4.3-1

THERMAL GRADIENT SELECTED TEMPERATURE OF
ADULT CABEZON (N=1)
ACCLIMATED AT 16 C

TABLE 4.3-1

MEAN SELECTED TEMPERATURES (MST)
OF 16 C ACCLIMATED CABEZON
IN THERMAL GRADIENTS

Experiment	Period 1			Period 2 (Reversed Gradient)		
	MST	Standard Deviation	N	MST	Standard Deviation	N
6ar	11.1	3.7	10	9.8	0.6	8
6as	15.9	1.2	11	13.7	2.6	8
6at	10.0	3.3	11	8.2	1.0	8
6au	17.9	3.2	10	11.0	4.8	8
6av	15.0	2.2	10	13.9	3.7	8
6bg	14.2	2.3	12	13.0	2.8	9
6bh	14.6	3.8	11	12.3	2.9	8
6bi	9.5	3.4	10	17.8	2.5	7
6bj	14.0	3.3	10	14.3	2.9	9
6bl	13.7	3.8	11	11.2	2.5	9
6bm	9.2	2.2	10	17.2	1.3	7

variable. The overall MST for all experiment periods was calculated to be 13.0 C.

The frequency of occurrence of the fish with respect to temperature is illustrated in FIGURES 4.3-2 through 4.3-5. These figures also show the overall fish frequencies by each of the major periods of the experiment (mid-point gradient reversals). The maximum and minimum MST values for the fish were 17.9 and 8.2 C. The temperature gradient patterns of fish distribution varied considerably among experiments and produced test results that were without consistent patterns. Examining all of the 1979 test results in a summary fashion, it can be stated that the fish preferred temperatures below 16 C (in most experiments avoided temperatures above 18 C) and were variably distributed throughout the gradient without distinct patterns other than the tendency towards the MST.

4.3.4 DISCUSSION

These results of the laboratory temperature gradient behavior tests will enable environmental assessments of the thermal discharge plume on the distribution of animal species in Diablo Cove. Many fish and some invertebrate species have been shown to respond significantly in their habitat selection to water temperatures. In this respect, there is a potential for some species to be thermally excluded from the discharge region (loss of habitat) or attracted to the discharge region.

In addition, these results of the temprature gradient behavior experiments were undertaken to gain information on the species' optimum temperature for growth. Based on experiments with freshwater fish species, it has been shown that there are examples of a direct relationship between preferred temperature and optimum temperature for growth.

The results of the cabezon temperature gradient behavior experiments have indicated a region of upper temperature avoidance. When integrated with measured or predicted thermal plume characteristics, an accounting of excluded

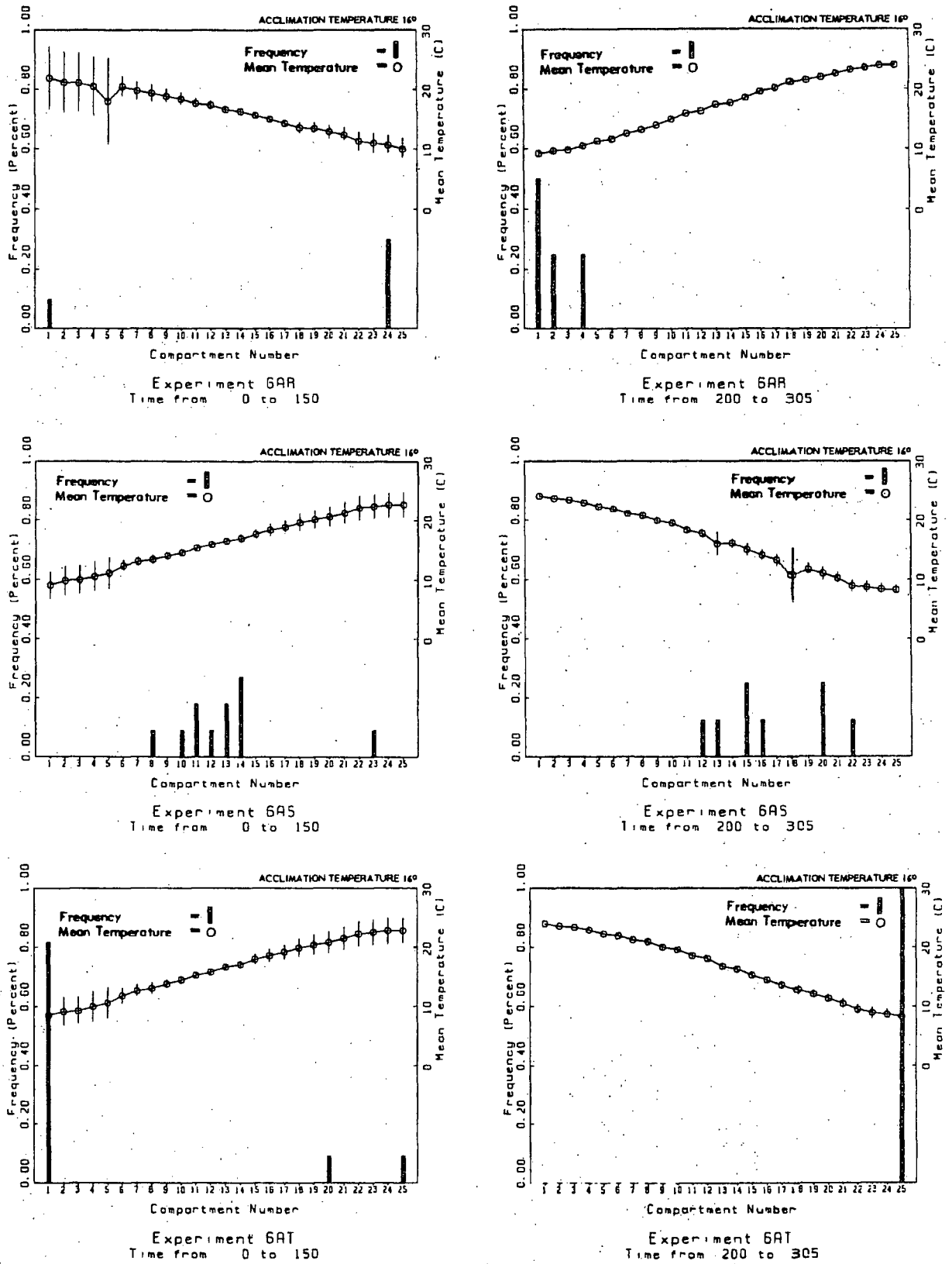


FIGURE 4.3-2

MEAN TEMPERATURE AND PERCENT FREQUENCY DURING TWO OBSERVATION PERIODS (REVERSED GRADIENT DIRECTIONS) OF ADULT CABEZON (16 C ACCLIMATION) BY THERMAL GRADIENT COMPARTMENT

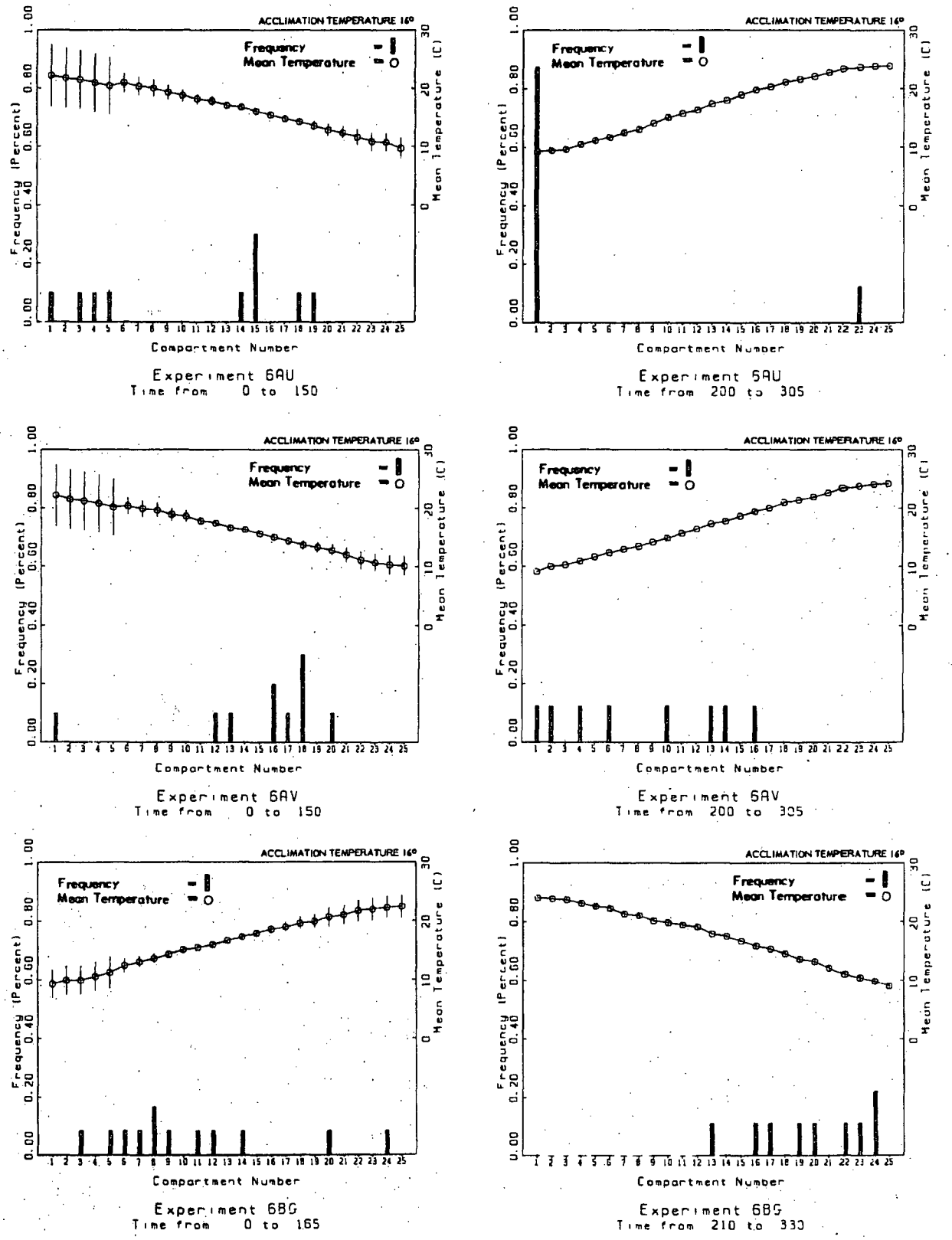


FIGURE 4.3-3

MEAN TEMPERATURE AND PERCENT FREQUENCY DURING TWO OBSERVATION PERIODS (REVERSED GRADIENT DIRECTIONS) OF ADULT CABEZON (16 C ACCLIMATION) BY THERMAL GRADIENT COMPARTMENT

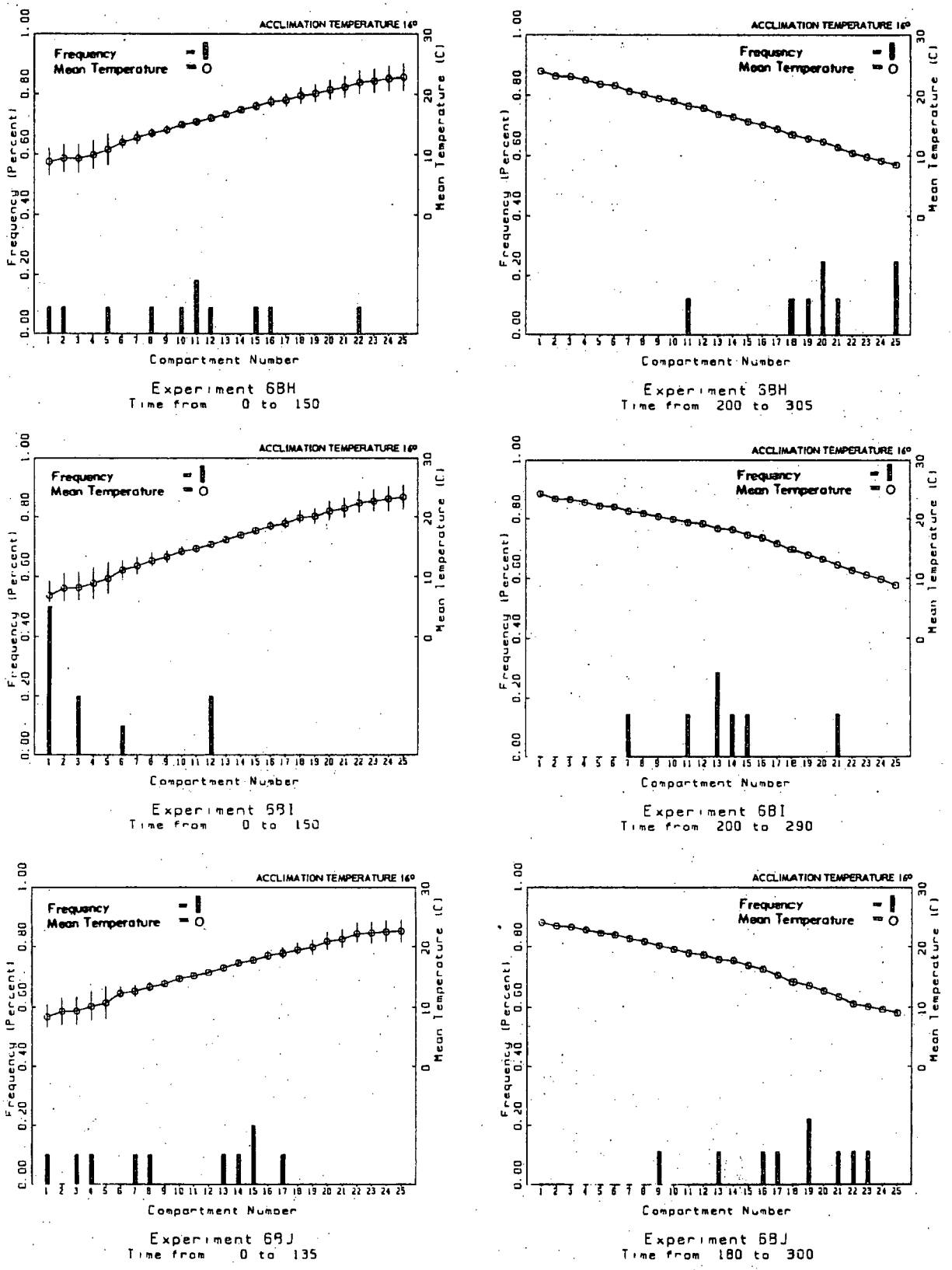


FIGURE 4.3-4

MEAN TEMPERATURE AND PERCENT FREQUENCY DURING TWO OBSERVATION PERIODS (REVERSED GRADIENT DIRECTIONS) OF ADULT CABEZON (16 C ACCLIMATION) BY THERMAL GRADIENT COMPARTMENT

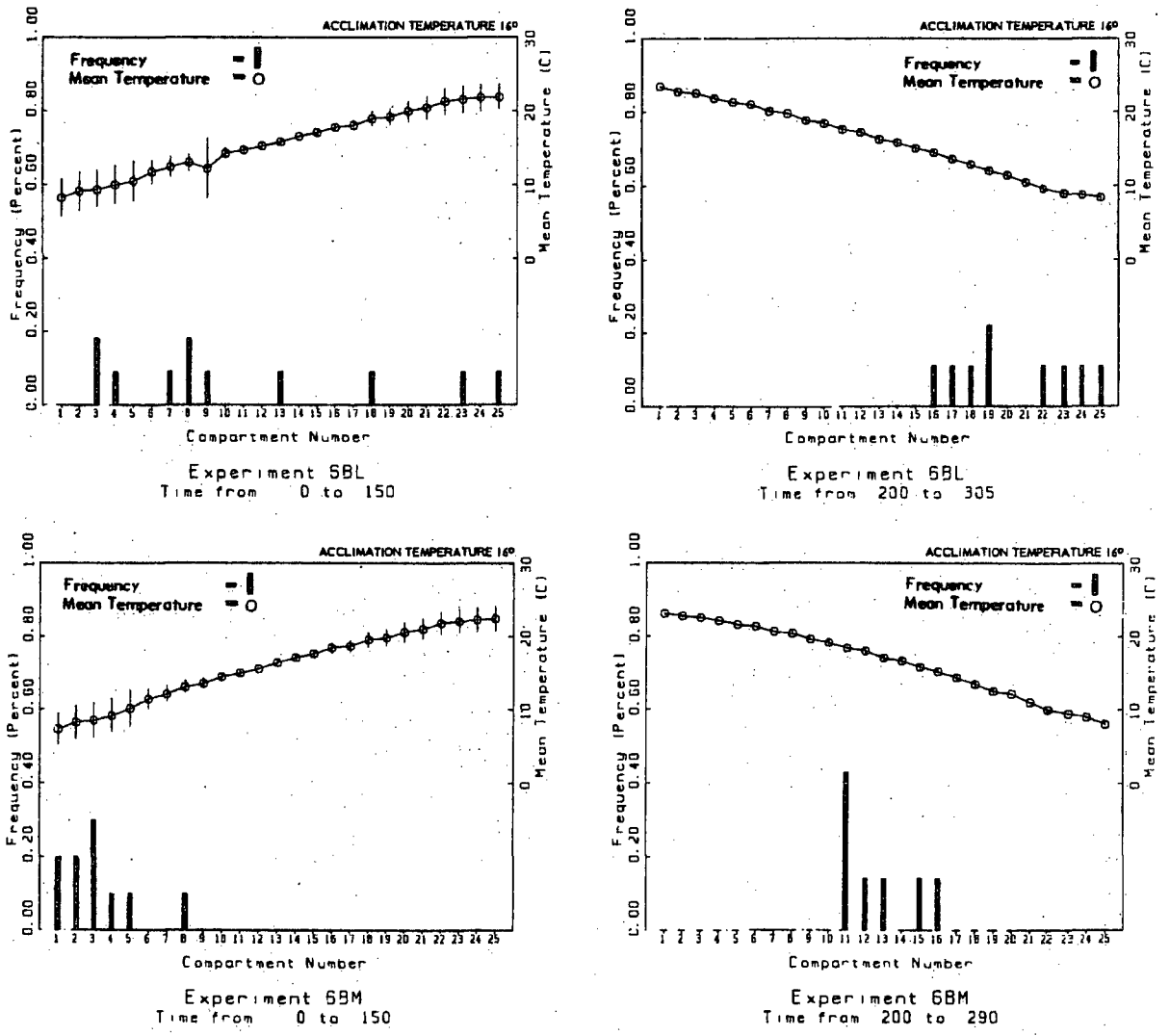


FIGURE 4.3-5

MEAN TEMPERATURE AND PERCENT FREQUENCY DURING TWO OBSERVATION PERIODS (REVERSED GRADIENT DIRECTIONS) OF ADULT CABEZON (16 C ACCLIMATION) BY THERMAL GRADIENT COMPARTMENT

habitat could possibly be estimated for a variety of power plant operating conditions.

The use of temperature preference results to estimate the optimum temperature for growth does not seem promising based on the species studied herein. From our findings we would predict that the optimum temperature for cabezon growth would be 13 C (55.4 F) for 16 C (61 F) acclimated fish. However, there was no cabezon growth data to compare with this estimate, and the wide variance of the estimate would seem to preclude its usefulness.

4.4 ROCK PRICKLEBACK (XIPHISTER MUCOSUS)

A series of behavioral experiments was designed to examine the thermal preference/avoidance response of selected fish and invertebrate species which are found in the Diablo Cove (discharge) area. This section contains our findings which were not previously reported for rock prickleback (Xiphister mucosus). The following experiments with rock prickleback include ten trials of five fish each trial, using fish acclimated at 16 C and 12 C.

The experimental apparatus and methods employed in the temperature gradient experiments are briefly summarized below. For additional procedural details, the reader is referred to the laboratory procedure included in APPENDIX B (Procedure 306, Rev. 2) and experimental protocols in APPENDIX C (experiments 6a, j, k, m, n, o, q, u, v) of this report. The apparatus employed in these experiments has been described in detail in an early progress report (PGandE 1977, Section 6.2.2.6).

4.4.1 SPECIES DESCRIPTION

The rock prickleback is one of eight species of the Family Stichaeidae found at Diablo Canyon. Three additional pricklebacks are found elsewhere in California. Two species of the genus Xiphister are present at Diablo Canyon: X. mucosus and X. atropurpureus.

This prickleback ranges from Port San Juan Alaska to Point Arguello boat station, Santa Barbara County, California, occurring from the intertidal to a depth of 60 ft (Miller and Lea 1972). This species is found under rocks from the upper intertidal zone, which is preferred by juveniles, to depths of 60 ft (Clemens and Wilby 1961, Hart 1973). During the Burge and Schultz (1973) studies at Diablo Canyon, all 169 rock prickleback were found at the shore stations (0-10 ft below MLLW). Warrick (pers. comm.) has found primarily immature rock prickleback in the intertidal region at Diablo Canyon.

4.4.2 METHODS

A sample of rock prickleback was collected from intertidal areas in the immediate vicinity of Diablo Cove. The specimens were collected by hand dip nets, placed in aerated tubs and transported to the Diablo laboratory. The fish were held in ambient (12 C) running seawater tanks and fed a maintenance diet of cut squid for an average period of approximately 60 days. During critical thermal maximum tests were performed on the population.

All of the collected fish were pooled and not kept separate by collection periods. Therefore some proportion of the fish (< 25 percent) were held in the laboratory longer than others, but the majority of the pooled test specimens were held for less than 60 days before their use in the thermal preference experiments. The effect of holding these fish in the laboratory is unknown; however, some temperature preference change may have occurred due to nutrition and ambient laboratory seawater temperatures which are lower than Morro Bay temperatures. During the holding period (minimum of 48 hours) any injured fish or fish displaying abnormal behavior (schooling, feeding, swimming and equilibrium) were removed from the test populations.

Following the observational holding period, the selected test specimens were transferred to acclimation containers and the water temperatures raised or lowered to the specified acclimation temperatures (16 C), at the rate of 1 C per day. The fish were held at these acclimation temperatures until acclimation was achieved as measured by no further change in thermal resistance in a series of daily critical thermal maximum (CTM) tests, according to Procedure 308. The results of the CTM tests were employed to establish that acclimation was occurring, but full acclimation was probably achieved in the additional 1- to 2-week holding period at acclimation temperatures following the CTM tests and prior to the preference tests.

The acclimated fish were carefully transferred by bucket to the gradient test chamber upon completion of the gradient chamber setup described in APPPENDIX B (Procedure 306). The pretest gradient temperature was established

uniformly throughout the gradient at ±0.5 C of the 16 C acclimation temperatures. Photoperiod (lighting timer switch) was set to 14-hour light and 10-hour dark beginning with a lighted condition.

Positions of the fish in the gradient are recorded by three overhead 35 mm cameras with strobe lights that are automatically triggered by an electronic timer. The gradient temperature is measured electronically in each of the 25 gradient chamber compartments by thermistors imbedded in the chamber floor. The compartment temperatures are recorded manually on a YSI 81A recorder and automatically on an Autodata Nine temperature logger at 60-minute intervals. Digital time displays are positioned in the gradient chamber so that they appear in each photograph along with the compartment numbers.

The temperature range in the gradient is controlled by heating and chilling units with inflows to four heat exchangers, two at the ends and two on the bottom of the gradient chamber. Between runs, the hot and cold water inflows can be reversed to test any end preference effects. The gradient chamber is equipped with air supply airstones in each compartment to provide aeration and vertical circulation to destratify the gradient water temperatures.

The start of an experimental run is initiated by introducing the test fish (a single individual) into the gradient at their pre-experimental acclimation temperatures. In the case of the rock prickleback the acclimation temperatures of 12 C and 16 C were used. The fish was left in this condition for a period of 15 to 17 hours. At the end of this time, the temperature controllers are set to -6 C acclimation temperature (ΔT) at the cold end, -3C ΔT, mid-gradient cold end, +3 C ΔT mid-gradient warm end, and +6 C ΔT warm end. The cameras and temperature recording systems are set to 5-minute intervals and operated at this interval for 60 minutes. At the end of this transition period, the recording rate is reset to 15-minute intervals. The gradient is then maintained for 120 minutes with no less than a 10 C difference between the hot and cold ends. After this stable gradient period, the gradients are routinely adjusted over the remaining course of the experiment in order to treat the chamber compartments with a range of temperatures and obviate compartment selections. This observation period

continued for a period of not less than 7 hours and up to 24 hours. In this present experiment, the observation period from the establishment of the gradient was continued for a period of 5 hours (less than 24 hours from the introduction of the fish into the gradient chamber) and the gradient direction was reversed midway in the observation period.

At the termination of the experiment, the fish were removed from the gradient chamber, anesthetized, weighed and measured and returned to the wild. The photographic films were developed and projected using three side-by-side projectors, and the compartment positions of the fish were recorded on data sheets for each photographic period. The recorded compartment temperatures at the time of each photograph were also entered on the data sheet and the data entered into the computer.

Mean selected temperatures for each recording interval were computed according to the formula:

$$MST = \frac{\sum X_i t_i}{\sum X_i}$$

where: MST = mean selected temperature, degrees C

X_i = number of fish in i^{th} compartment

t_i = temperature of ith compartment (degrees C)

i = 2 . . . 24 compartment with fish

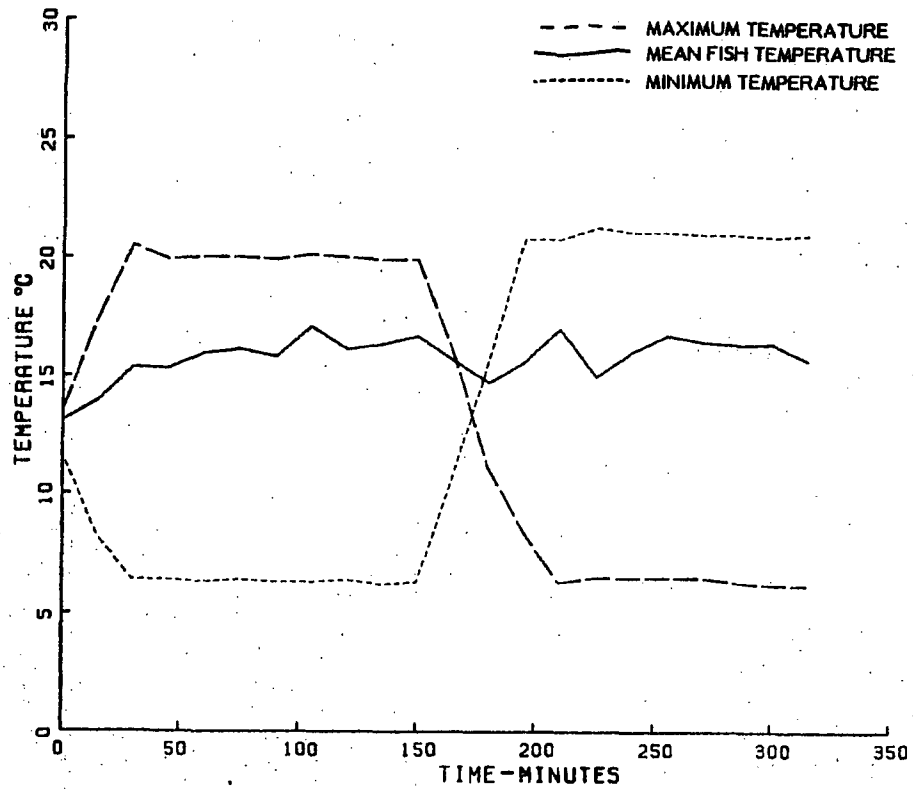
The fish and temperatures of the end compartments (compartments 1 and 25) were excluded from the calculation to obviate suspected positional effects on selection behavior. Overall MST values were also calculated separately for each half of the experiment (reversed gradient periods).

4.4.3 RESULTS

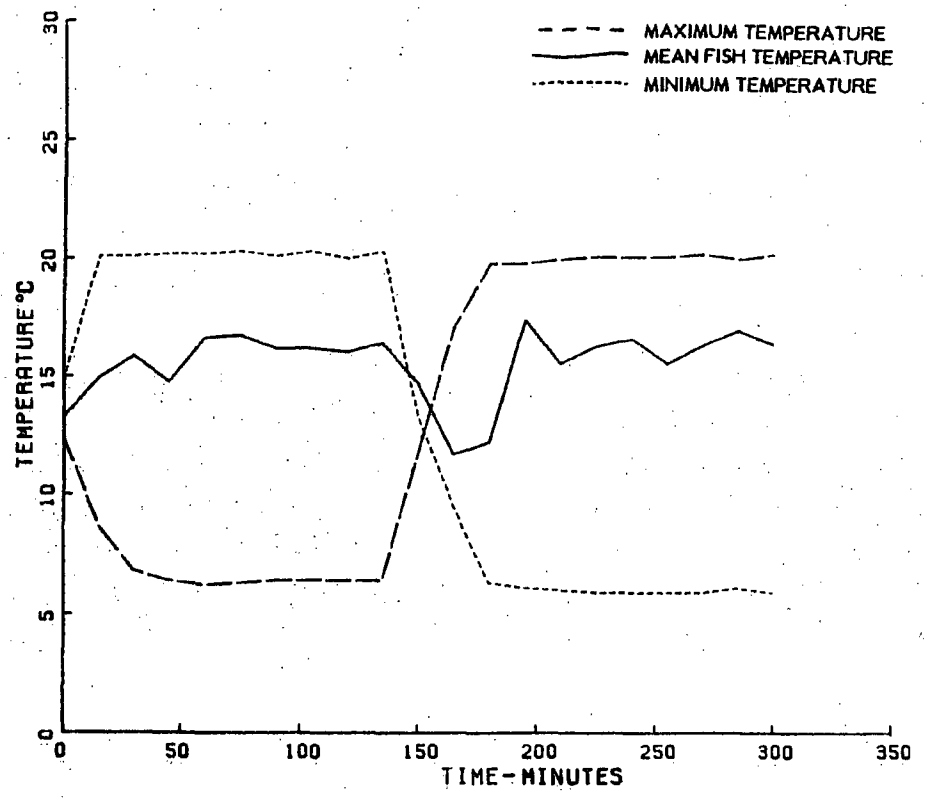
Results of the thermal gradient preference tests for rock prickleback acclimated at 12 C and 16 C are expressed as the MST. The MST values for the 12 C and 16 C acclimated fish were summarized by recording periods during the course of the experiment and are shown in examples in FIGURES 4.4-1 and 4.4-2, respectively. The experiment was broken into an initial 6-hour period characterized by minimum thermal gradient. In practice a small gradient was present during this period due to the need to heat and chill in order to maintain a stable temperature (acclimation temperature).

Following this 6-hour period of gradient chamber acclimation, an experimental temperature gradient was established by increasing the heating and cooling at opposite ends of the gradient. Observations were made at five-minute intervals for 60 minutes during this transition to a wide range gradient. In the case of rock prickleback, the immediate response to the gradient was different for the 12 C and 16 C acclimated fish. As seen in other species tested at the laboratory, the 16 C fish sought lower gradient temperatures and the 12 C fish sought higher temperatures. The MST of the rock pricklebacks tested were relatively stable and constant throughout the experiment compared to other species tested. Of all the fish species tested, the rock prickleback appears to be the most plastic in its thermal behavioral response based on the close agreement between acclimation temperature and mean selected temperature. The MST values of the fish were very stable throughout the experiment compared to other species tested.

The MST values were calculated for each experimental period (two periods per experiment, reversed gradient) and are summarized in TABLE 4.4-1. MST variance, shown as standard deviation in TABLE 4.4-1, was relatively uniform throughout the experiments; and, as noted above, the MST values are much less variable than other species tested. The overall MST for all experiment periods was calculated to be 16.0 C for 12 C acclimated fish and 13.6 C for 16 C acclimated fish.



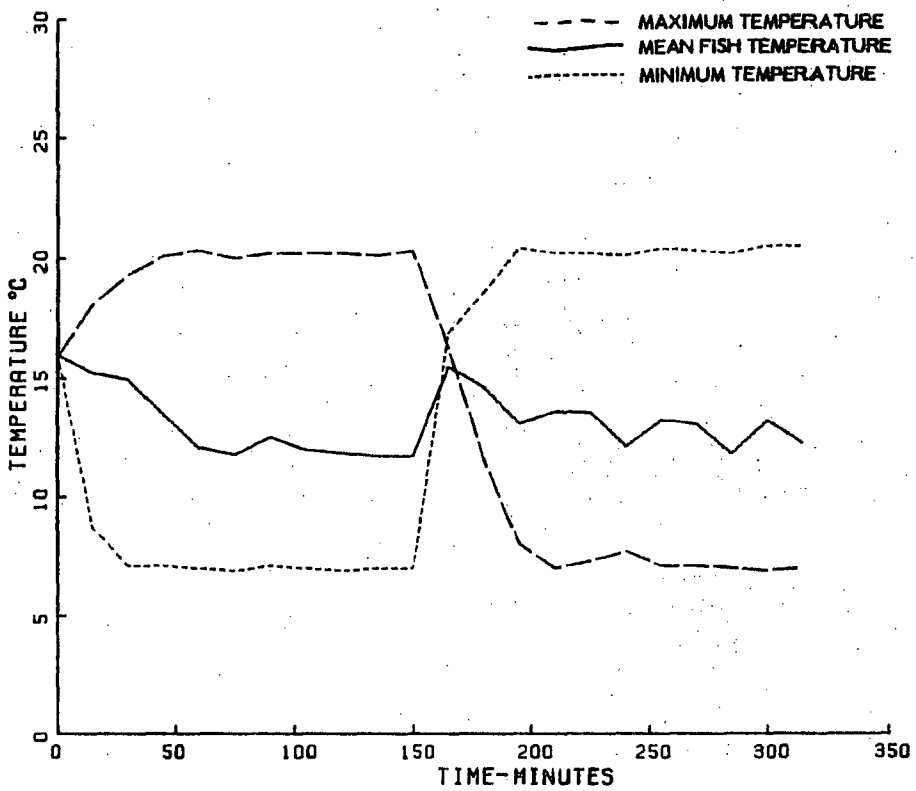
EXPERIMENT = 6BE



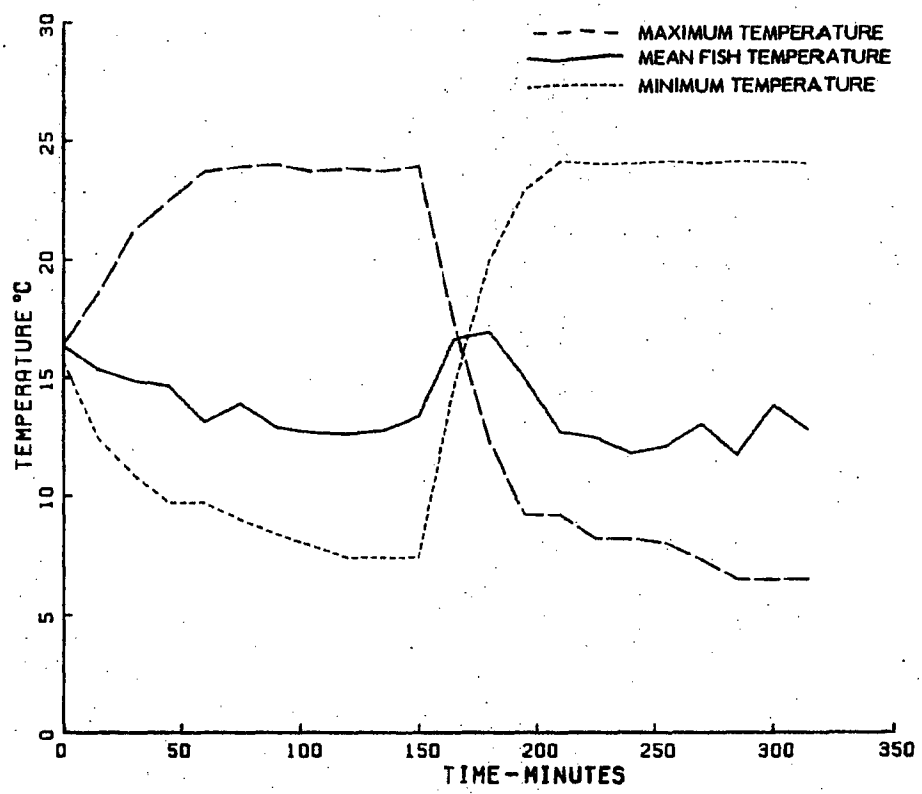
EXPERIMENT = 6AY

FIGURE 4.4-1

THERMAL GRADIENT MEAN SELECTED TEMPERATURE VS. TIME OF JUVENILE ROCK PRICKLEBACK FISH (N=5) ACCLIMATED AT 12 C



EXPERIMENT = 6BC



EXPERIMENT = 6AW

FIGURE 4.4-2

THERMAL GRADIENT MEAN SELECTED TEMPERATURE VS. TIME OF JUVENILE ROCK PRICKLEBACK FISH (N=5) ACCLIMATED AT 16 C

TABLE 4.4-1

MEAN SELECTED TEMPERATURE (MST) OF 12 C AND 16 C ACCLIMATED ROCK PRICKLEBACK IN THERMAL GRADIENT EXPERIMENTS

Experiment Code Number	Acclimation Temperature (°C)	Period 1			Period 2 (Gradient Reversal)		
		MST (°C)	Standard Deviation	Number of Observations	MST (°C)	Standard Deviation	Number of Observations
6aw	16	13.9	3.3	55	12.5	2.4	40
6ax	16	11.0	3.8	52	15.3	3.6	40
6ay	12	15.7	1.7	49	15.9	2.8	45
6az	12	13.6	3.1	50	15.1	2.6	45
6ba	12	16.5	1.9	59	17.6	1.5	40
6bb	12	18.9	0.6	48	14.8	3.3	45
6bc	16	12.9	2.1	54	12.8	1.6	40
6bd	16	16.0	3.3	55	14.6	1.5	40
6be	12	15.7	2.9	54	16.2	3.6	40
6bf	16	13.0	3.5	110	14.1	2.4	80

The frequency of occurrence of the fish with respect to temperature is illustrated in FIGURES 4.4-3 through 4.4-6. These figures also show the overall fish frequencies by each of the major periods of the experiment (mid-point gradient reversals). The maximum and minimum MST values for the fish were 18.9 C and 13.6 C for 12 C acclimated fish and 16.0 C and 11.0 C for 16 C acclimated fish. The temperature gradient patterns of fish distribution varied considerably among experiments and produced test results that were without consistent patterns. Examining all of the 1979 test results in a summary fashion, it can be stated that the fish preferred temperatures below 16 C (in most experiments avoided temperatures above 18 C) and were variably distributed throughout the gradient without distinct patterns other than the tendency towards the MST.

4.4.4 DISCUSSION

These results of the laboratory temperature gradient behavior tests will enable environmental assessments of the thermal discharge plume on the distribution of animal species in Diablo Cove. Many fish and some invertebrate species have been shown to respond significantly in their habitat selection to water temperatures. In this respect, there is a potential for some species to be thermally excluded from the discharge region (loss of habitat) or attracted to the discharge region.

In addition, these results of the temprature gradient behavior experiments were undertaken to gain information on the species' optimum temperature for growth. Based on experiments with freshwater fish species, it has been shown that there are examples of a direct relationship between preferred temperature and optimum temperature for growth.

The results of the rock prickleback temperature gradient behavior experiments have indicated a region of upper temperature avoidance. When integrated with measured or predicted thermal plume characteristics, an accounting of excluded habitat could possibly be estimated for a variety of power plant operating conditions.

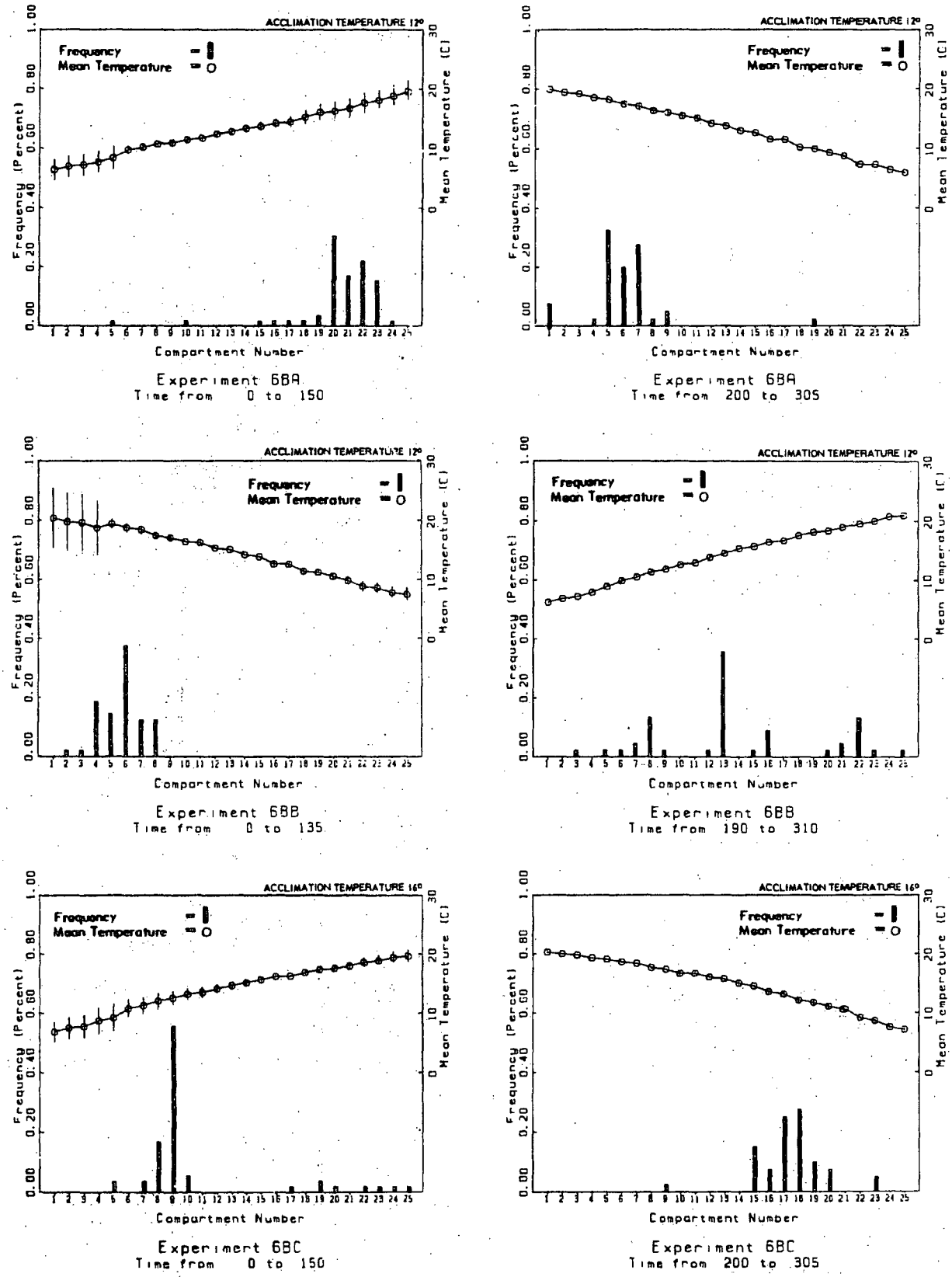


FIGURE 4.4-3

MEAN TEMPERATURE AND PERCENT FREQUENCY DURING TWO OBSERVATION PERIODS (REVERSED GRADIENT DIRECTIONS) OF JUVENILE ROCK PRICKLEBACK FISH BY THERMAL GRADIENT COMPARTMENT

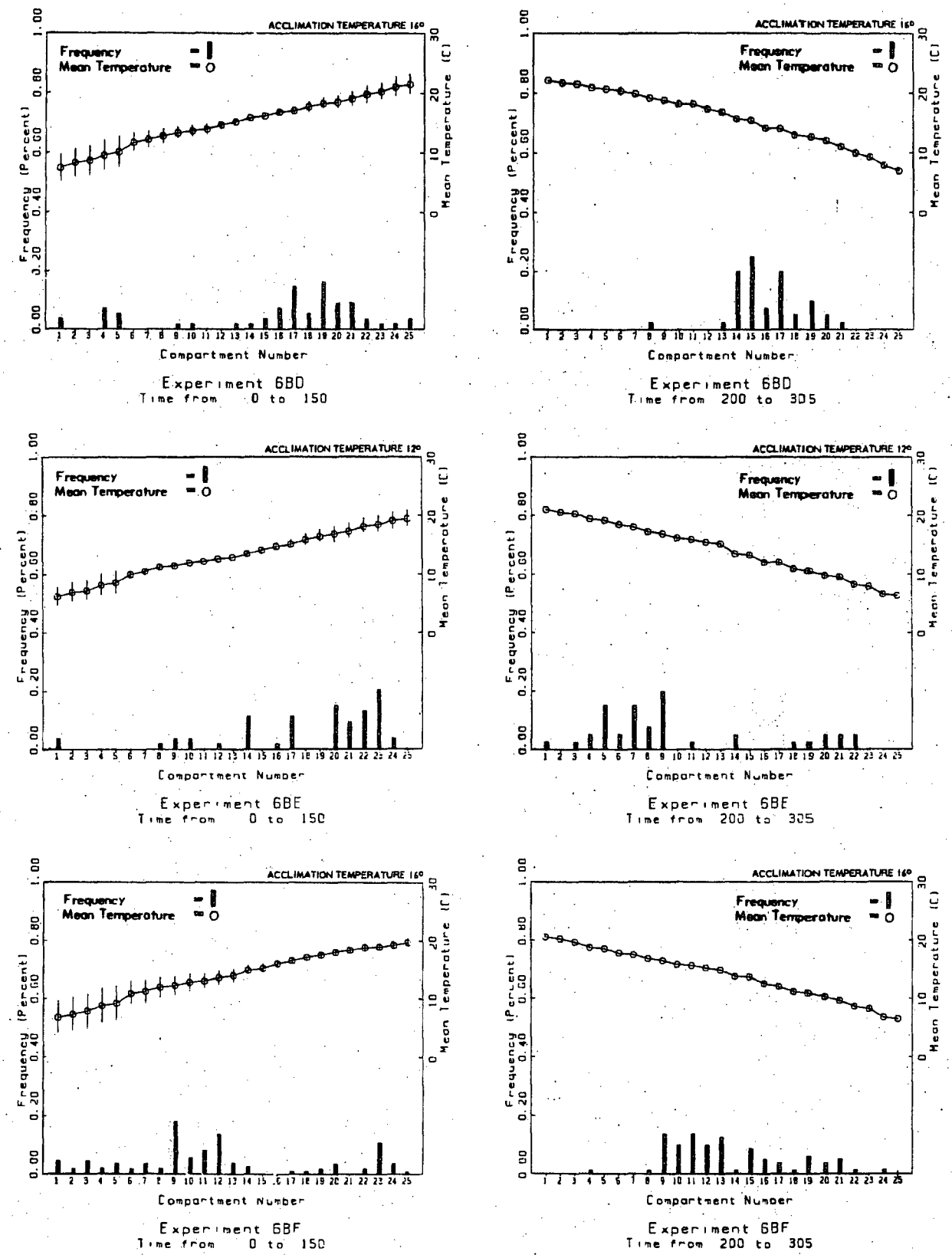


FIGURE 4.4-4

MEAN TEMPERATURE AND PERCENT FREQUENCY DURING TWO OBSERVATION PERIODS (REVERSED GRADIENT DIRECTIONS) OF JUVENILE ROCK PRICKLEBACK FISH BY THERMAL GRADIENT COMPARTMENT

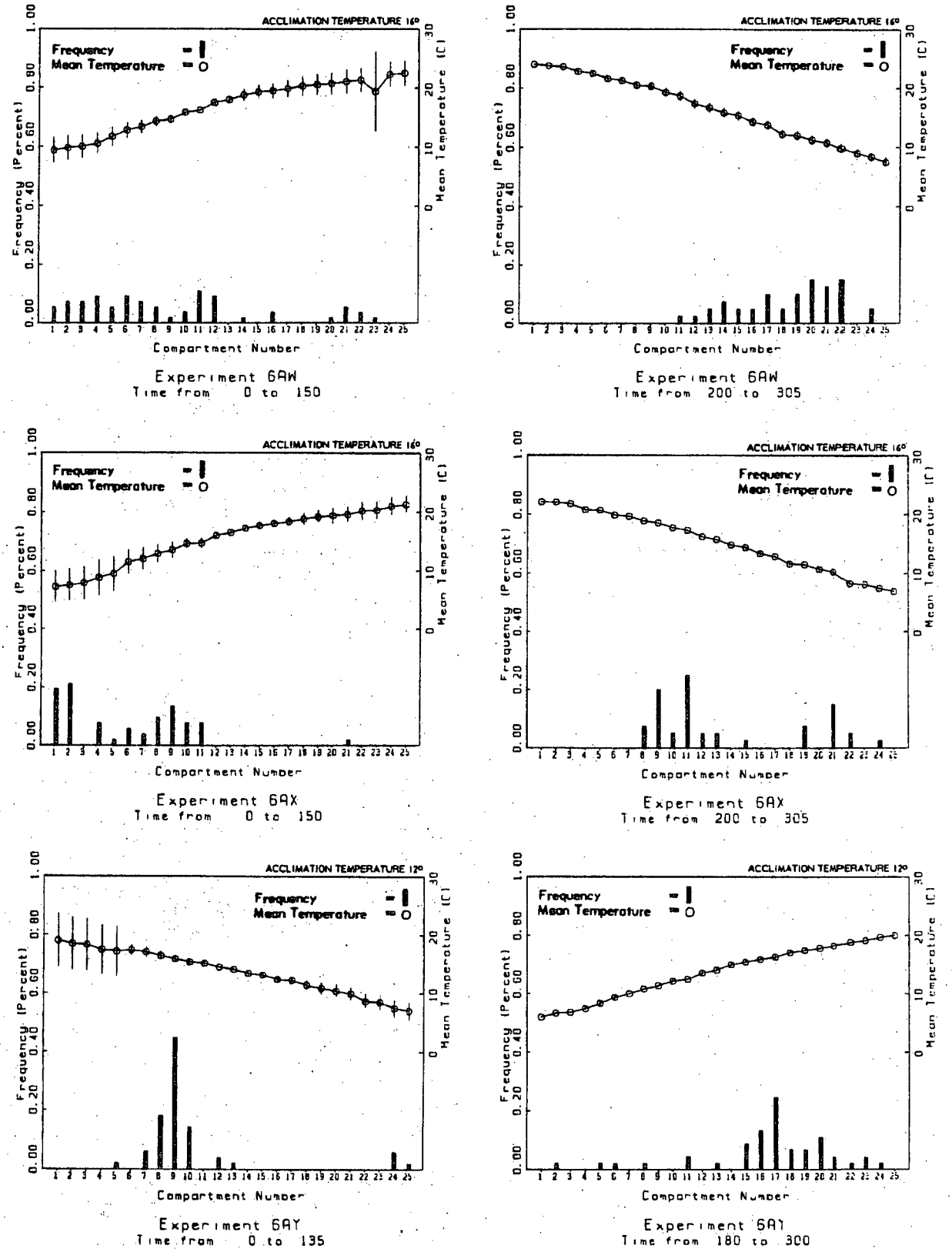
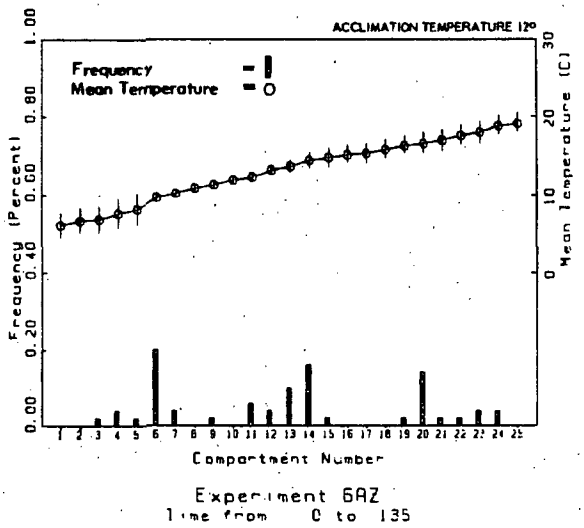


FIGURE 4.4-5

MEAN TEMPERATURE AND PERCENT FREQUENCY DURING TWO OBSERVATION PERIODS (REVERSED GRADIENT DIRECTIONS) OF JUVENILE ROCK PRICKLEBACK FISH BY THERMAL GRADIENT COMPARTMENT



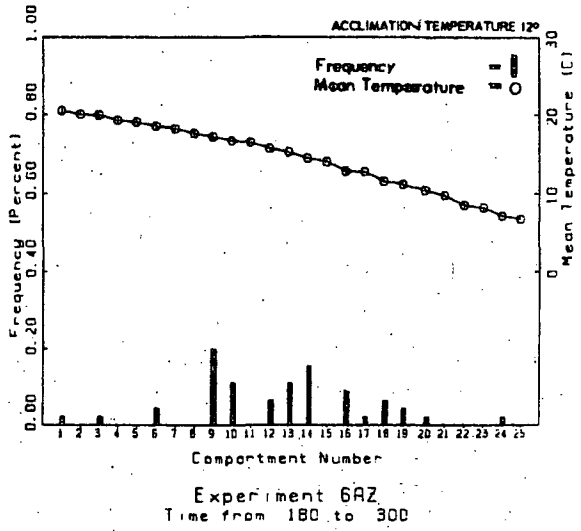


FIGURE 4.4-6

MEAN TEMPERATURE AND PERCENT FREQUENCY DURING TWO OBSERVATION PERIODS (REVERSED GRADIENT DIRECTIONS) OF JUVENILE ROCK PRICKLEBACK FISH BY THERMAL GRADIENT COMPARTMENT

The use of temperature preference results to estimate the optimum temperature for growth does not seem promising based on the other species studied herein nor with the rock prickleback. From our findings we would predict that the optimum temperature for rock prickleback growth would be 16.0 C (61 F) for 12 C (54 F) acclimated fish and 13.6 C (56.5 F) for 16 C (61 F) acclimated fish. Laboratory growth data for rock prickleback demonstrated an optimum temperature for growth range of 14-18 C (57.2-64.4 F), a mid-point of 16 C (61 F). These results were based on fish which had been held (acclimated) in ambient laboratory seawater of 14 C (57.2 F). Given the range of optimum growth temperatures and the MSTs it is difficult to establish any consistent relationship between the rock prickleback's MST and its optimum temperature for growth.

4.5 BLACK ABALONE (HALIOTIS CRACHERODII) THERMAL PREFERENCE/AVOIDANCE

According to Morris et al. (1980), the black abalone (Haliotis cracherodii), is common along the California coast and found under large rocks and in crevices from the high intertidal region (where it is most abundant) to a 6 m depth. Its geographical distribution extends from Coos Bay, Oregon to Cabo San Lucas, Baja California, Mexico. It reaches a maximum length of over 20 cm.

Black abalone was designated a representative important species (RIS) for the Diablo Canyon 316(a) demonstration. The literature on black abalone was reviewed in a previous report (PGandE 1979b).

The objective of the thermal preference/avoidance experiments was to provide information on the behavioral response of black abalone to a thermal gradient, specifically, whether it exhibits preference for or avoidance to a particular temperature. The purpose was to provide information about the possible response of the species to the thermal plume, in particular, whether sublethal temperatures would have a major effect on its distribution in Diablo Cove.

4.5.1 METHODS

A summary of the methods employed in the thermal preference/avoidance experiments on black abalone is presented below. A more detailed account of the experimental procedure used in this experiment is presented in APPENDIX B (Procedure 317). The protocols for this experiment are included in APPENDIX C of this report (experiments 17b to i).

The adult specimens of black abalone used in this experiment were greater than 60 mm in length and were collected during late 1978 and early 1979 from the vicinity of Diablo Cove, using standard abalone irons.

A total of nine runs were made on this species. A summary of the run data is presented in TABLE 4.5-1. While being held in the laboratory prior to

TABLE 4.5-1

PROTOCOL SUMMARY FOR BLACK ABALONE
THERMAL PREFERENCE/AVOIDANCE

Run	Sample Size	Acclimation Temperature	Gradient Status (Temperature, C)		
			Initial	First Change	Second Change
17a[a]	25	Ambient	10-15	12	7-18
17b[a]	25	"	6-21	----	----
17c[b]	30	"	8-29	8-22	----
17d[b]	23	"	7-30	----	----
17e[b]	30	"	Ambient	8-26	13-17
17f[c]	30	"	8-30	13-14	----
17g[c]	30	18	7-31	----	----
17h[c]	30	18	8-37	18	----
17i[c]	31	21	7-37	21	----

[a] Run made with *Tegula brunnea* also in the tank.

[b] Run made with *Tegula brunnea* and *Haliotis rufescens* also in the tank.

[c] Run made with *Tegula brunnea*, *T. funebralis* and *Haliotis rufescens* also in the tank.

experimentation, the animals were fed to excess with Macrocystis and maintained in ambient temperature 4x8 ft flow-through tanks. During the holding period, the animals 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 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.

The 3-m gradient apparatus used for the experiment is described in detail in APPENDIX A. A brief description of the equipment is given here for the reader's convenience.

The 3-m gradient tank is designed to produce a horizontal thermal gradient to assess behavioral response of various large motile organisms. The gradient apparatus consists of a 380-cm-long tank with two primary heating and cooling chambers (40 x 60 x 45 cm) at both ends of the experimental chamber (300 x 20 x 45 cm). Eleven pairs of surface and bottom baffles divide the experimental chamber into 12 compartments. These baffles impede thermal convection currents and are capable of being raised or lowered to accommodate varying sized organisms and water depth. Gentle aeration from the bottom of each compartment eliminates any vertical stratification within compartments.

Heating and cooling is accomplished primarily through two stainless steel coils located in the end compartments. Two additional heat exchangers of Teflon coated stainless steel run along the bottom from the ends to the center of the experimental chamber and serve to produce a more uniform temperature gradient. Nylon netting at the ends and along the bottom of the experimental chamber prevents test organisms from entering the heating and cooling chambers and also provides a flat surface over the bottom baffles. Heat is supplied to two of the coils by a 42-gallon hot water heater. A 12-ton chiller cools a slump box which in turn supplies the coolant to the two cold coils. The heatant and coolant are circulated in a closed system by four pumps which are operated by four YSI

temperature controllers. A series of piping and control valves allow adjustment of the gradient and the switching of the hot and cold ends of the gradient between experiments. Flow meters are installed in line to facilitate precise temperature control.

The 12 physical compartments are subdivided into 25 observational compartments, in each of which is centered a thermistor probe. Temperatures are recorded on paper tapes by an Autodata Nine computer at predetermined time intervals.

Visual observations were made to determine the presence of the animals in each compartment of the experimental tank. Simultaneous temperature measurements were taken by the Autodata Nine computer and were later combined with the visual observations on one data sheet. The data were keypunched onto magnetic tape and presented graphically by an automatic plotting package.

Because these experiments represented the first attempts to study the behavioral responses of marine invertebrates to a thermal gradient, there were no standard experimental protocols to follow. From the initial runs, it was determined that the most efficient method of conducting the experiment was to establish the experimental temperature gradient in the experimental tank and to initiate the run by placing the test population in the compartment at the acclimation temperature for the test organisms. The organisms were then allowed to disperse in the tank for a period of hours without changing the temperature gradient. After at least one observation, recording of the position of the animals was done, the gradient was discontinued and the entire tank was maintained at the acclimation temperature of the organisms. Again after a period of hours, a final observation was made to verify that the animals could disperse throughout the tank. To a certain extent, the results presented reflect trials of various protocols used in determining the best approach. Once the experimental protocol was established, groups of animals acclimated to different temperatures were subjected to testing to determine the effects of acclimation on temperature avoidance.

It was possible to include more than one species simultaneously in the invertebrate thermal avoidance experiments, and this was done to the extent possible to increase efficiency. To be grouped together, species had to be compatible with each other (not prey or predators) and of a size and mobility that precluded physical obstruction of movement in the tank.

4.5.2 RESULTS

A summary of the protocols for the runs on black abalone was presented in TABLE 4.5-1. The data are presented in the graphs shown in FIGURES 4.5-1 to 4.5-6.

4.5.2.1 RUN 17B

The first run on this species was preliminary and involved placing the animals into an already established thermal gradient of 6-22 C. By 22 hours into the experiment, the animals had moved to the ends of the tank, where they remained until termination of the experiment (hour 65, FIGURE 4.5-1). It was concluded from this run that black abalone exhibited a strong tendency to aggregate in the corners of the tank and that the temperatures had not been sufficiently high to modify their normal behavior.

4.5.2.2 RUN 17C

The test animals were again introduced into an established thermal gradient in the second run on this species, but the temperature range was extended to 7-30 C (FIGURE 4.5-2). At 16 hours into the run, the animals had dispersed over that portion of the tank in which the temperature was at or below 21 C. After the 16-hour observation, the gradient range was reduced to 8-22 C, and at 40 hours the animals had dispersed across the entire tank.

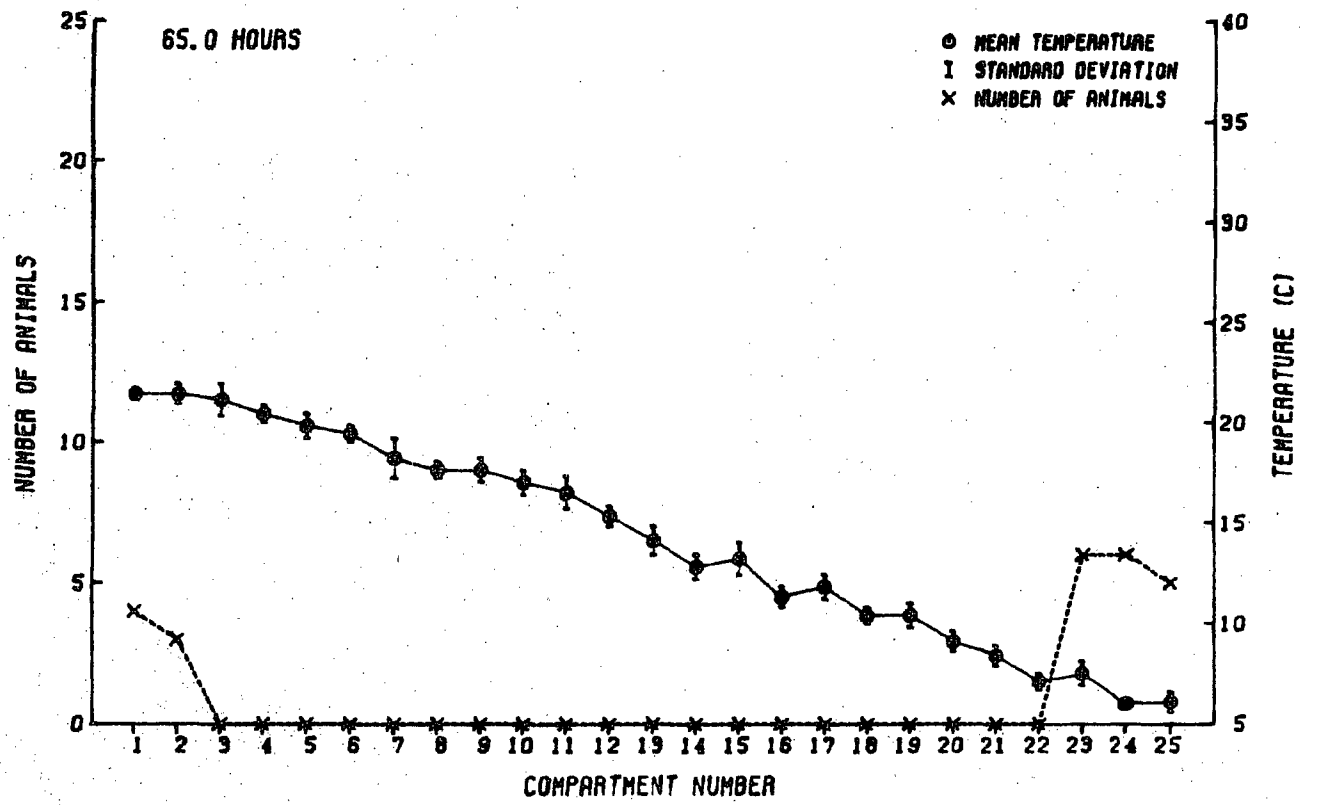


FIGURE 4.5-1

NUMBER OF ANIMALS AND TEMPERATURE VERSUS COMPARTMENT FOR AMBIENT-ACCLIMATED ANIMALS (RUN 17B): BLACK ABALONE THERMAL PREFERENCE/AVOIDANCE

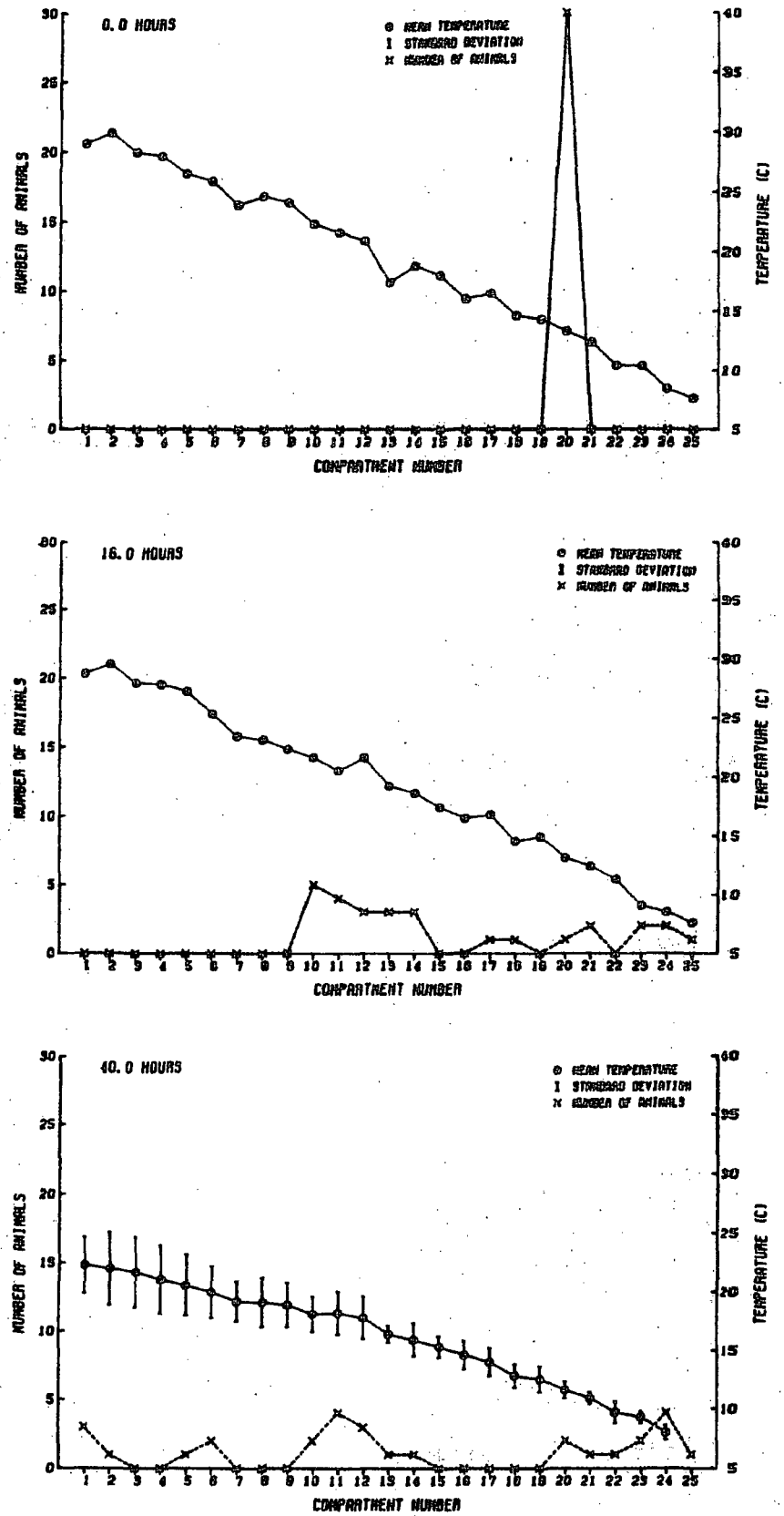


FIGURE 4.5-2

NUMBER OF ANIMALS AND TEMPERATURE VERSUS COMPARTMENT FOR AMBIENT-ACCLIMATED ANIMALS (RUN 17C): BLACK ABALONE THERMAL PREFERENCE/AVOIDANCE

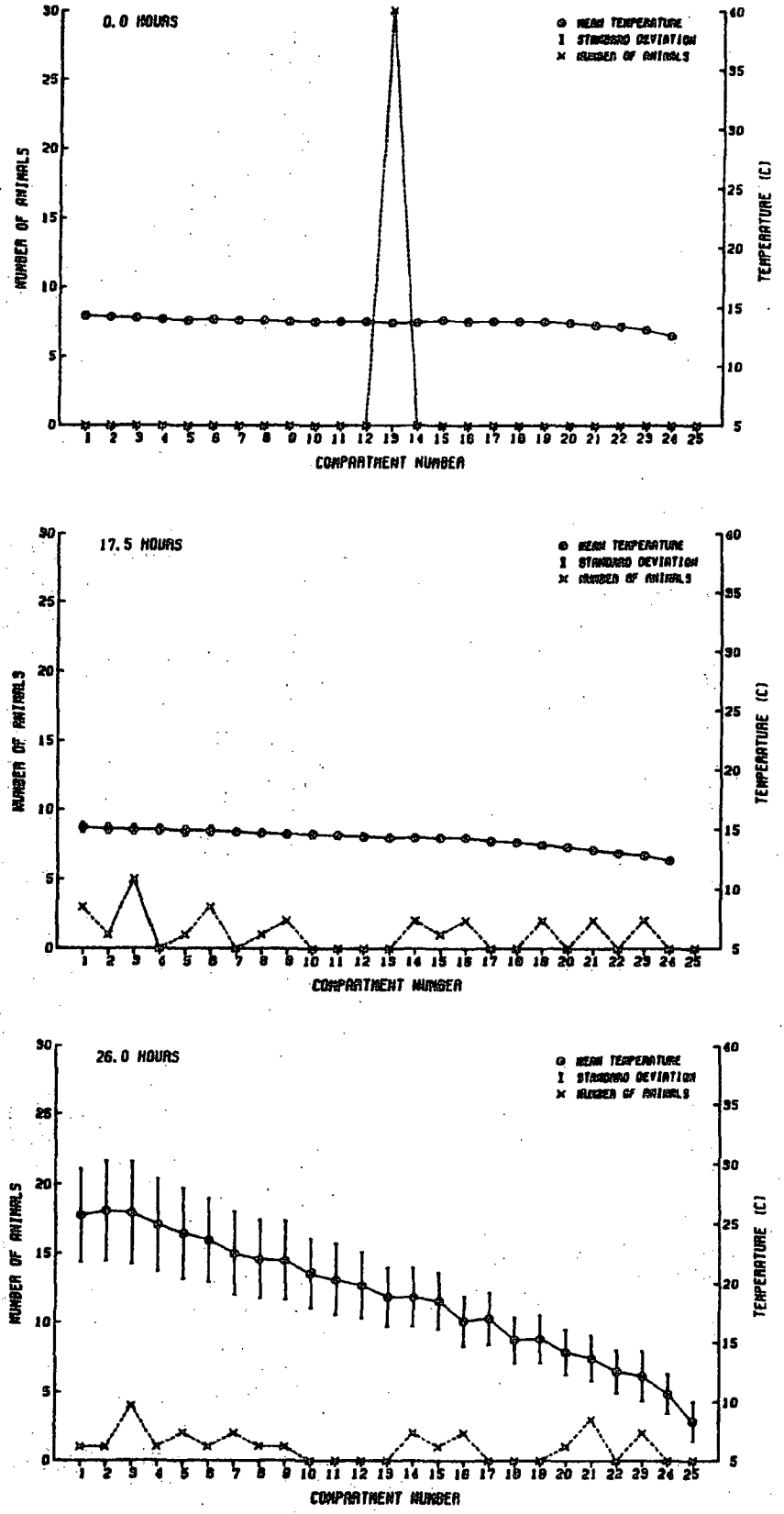


FIGURE 4.5-3

NUMBER OF ANIMALS AND TEMPERATURE VERSUS COMPARTMENT FOR AMBIENT-ACCLIMATED ANIMALS (RUN 17E): BLACK ABALONE THERMAL PREFERENCE/AVOIDANCE

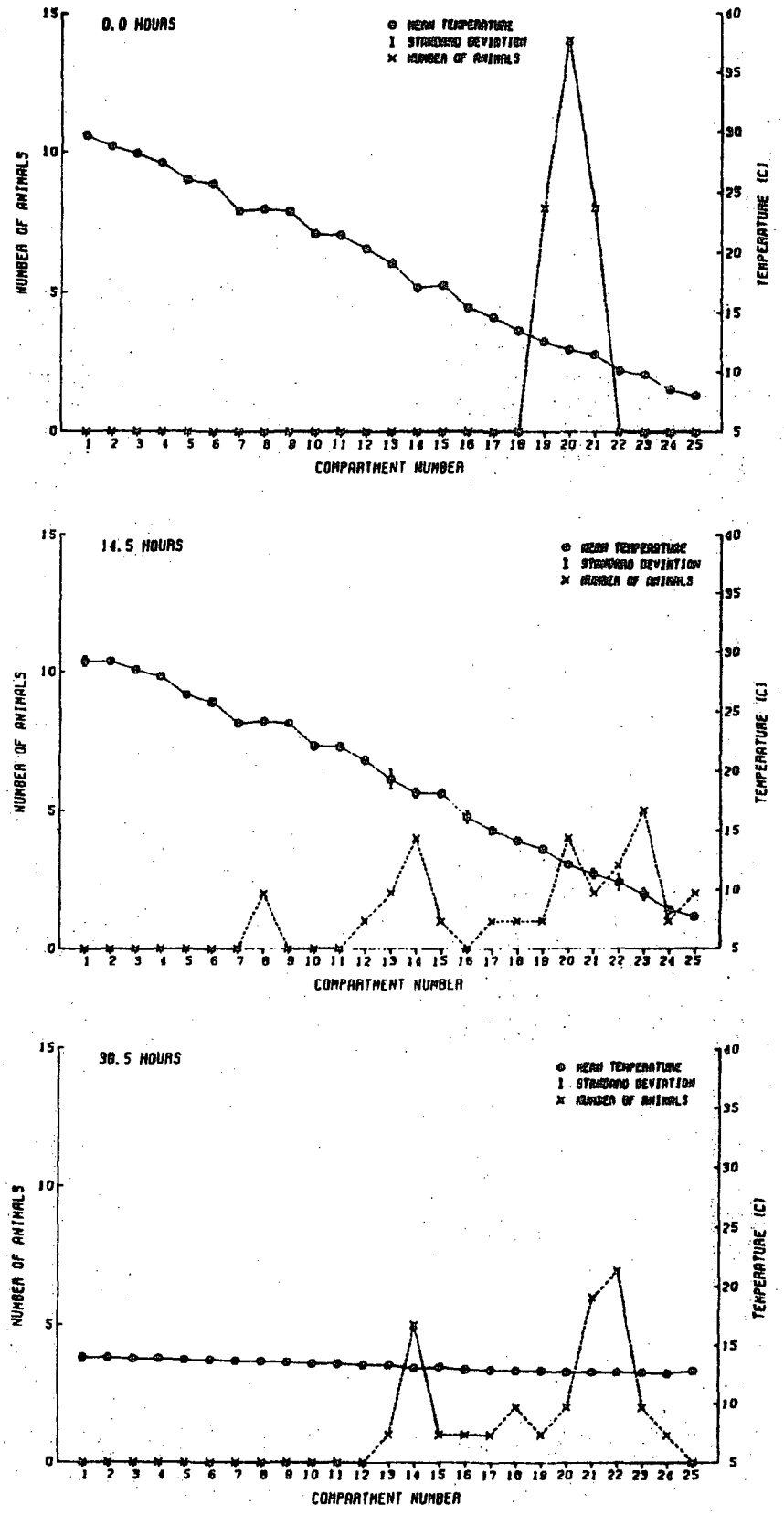


FIGURE 4.5-4

NUMBER OF ANIMALS AND TEMPERATURE VERSUS COMPARTMENT FOR AMBIENT-ACCLIMATED ANIMALS (RUN 17F): BLACK ABALONE THERMAL PREFERENCE/AVOIDANCE

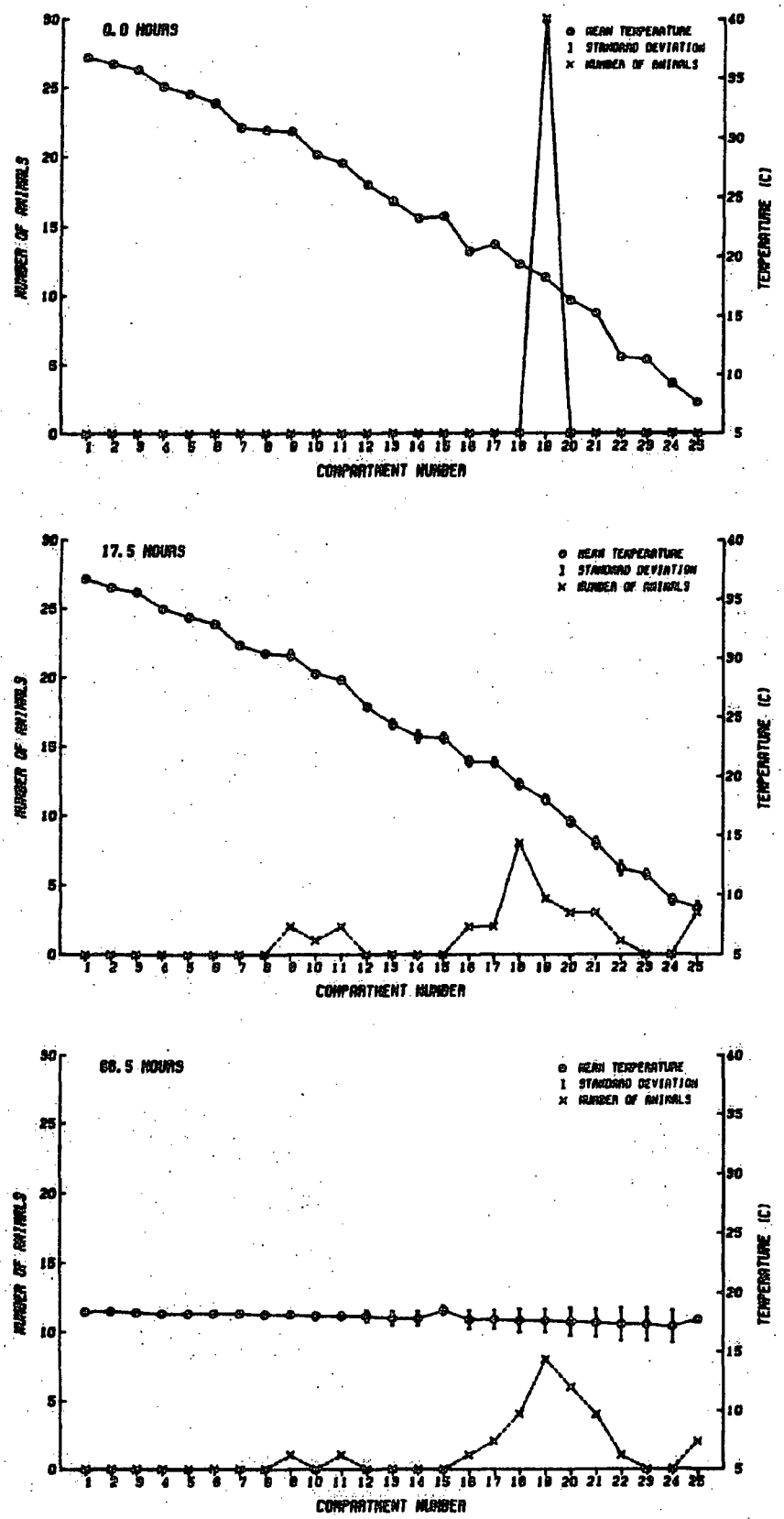


FIGURE 4.5-5

NUMBER OF ANIMALS AND TEMPERATURE VERSUS COMPARTMENT FOR AMBIENT-ACCLIMATED ANIMALS (RUN 17H): BLACK ABALONE THERMAL PREFERENCE/AVOIDANCE

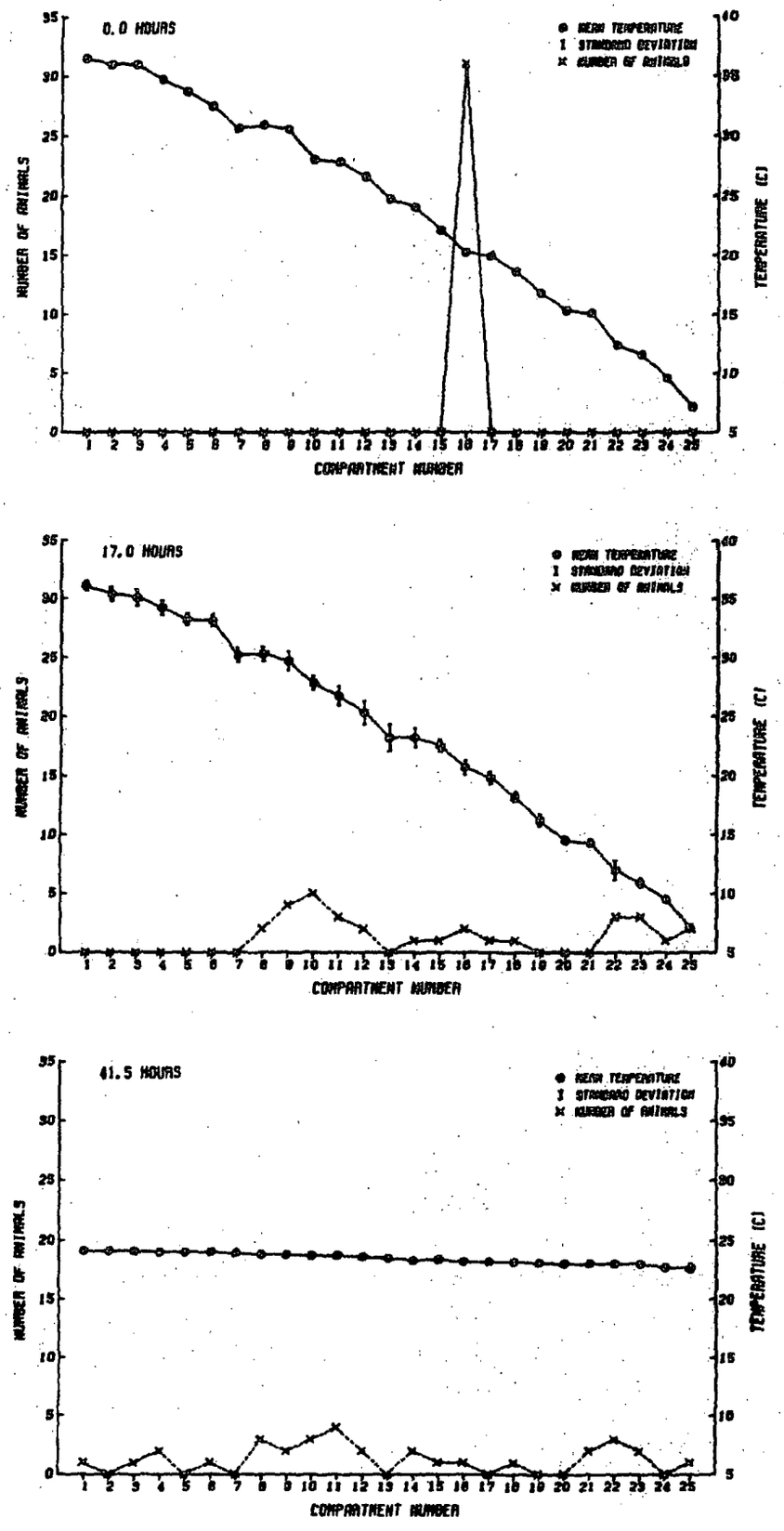


FIGURE 4.5-6

NUMBER OF ANIMALS AND TEMPERATURE VERSUS COMPARTMENT FOR AMBIENT-ACCLIMATED ANIMALS (RUN 171): BLACK ABALONE THERMAL PREFERENCE/AVOIDANCE

4.5.2.3 RUN 17E

The animals were introduced into compartment 13 with the tank at about 13 C (no thermal gradient) and by 17.5 hours had dispersed over the entire tank (FIGURE 4.5-3). A gradient of 8-26 C was then induced, and at the 26-hour observation this gradient had not significantly affected their distribution in the tank. The gradient was then reduced to 13-18 C, and at the 40-hour observation the distribution of the animals remained essentially the same.

4.5.2.4 RUN 17F

The animals were introduced into the tank with a gradient already established (8-29 C). By 14.5 hours, the animals had dispersed over the tank up to 24 C (FIGURE 4.5-4). However, when the gradient was subsequently reduced to 13-14 C, by 38.5 hours the animals did not redistribute themselves over the portion of the tank that was previously excluded.

4.5.2.5 RUN 17G

Prior to this run, all animals had been ambient acclimated. In this run, 18 C-acclimated animals from the snail growth experiment were placed at their acclimation temperature within a range of 7-31 C. At 12 hours, they had dispersed over the whole tank.

4.5.2.6 RUN 17H

This run involved a second test of the 18 C-acclimated animals. A 7-37 C gradient was established prior to placing the animals in the tank. By 17.5 hours the animals had dispersed over the tank up to 30 C (FIGURE 4.5-5). One of the two animals that moved beyond 29 C died. The gradient was subsequently reduced to 17-18 C but by 66.5 hours the animals had not moved into compartments not occupied at 17.5 hours.

4.5.2.7 RUN 171

In this final run on black abalone, 21 C-acclimated animals from the snail growth experiment were introduced into the tank with a 7-36 C gradient. At the 17.5 hour observation, they had dispersed across the tank up to 30 C (FIGURE 4.5-6). The gradient was subsequently reduced to 23-24 C and by 41.5 hours the animals had dispersed across the entire tank.

4.5.3 DISCUSSION

In general, the black abalone tested in this series of experiments exhibited neither an escape nor a preference response to temperature. An avoidance response was consistently evident, however, in that the animals did not move into locations where the temperature exceeded a certain value. This "avoidance temperature" was positively related to the acclimation temperature of the animals prior to testing (21-24 C (69.8-75.2 F) for ambient acclimated and 30 C (86 F) for 18-21 C- (64.4-69.8 F-) acclimated animals). One mortality was observed in an animal which had moved up to the avoidance temperature of 30 C (86 F). A strong tendency of the animals to cluster at the ends of the tank in an initial run could have resulted from either a thigmotactic (touching) response or a lack of turning response after encountering the end wall. The absence of end wall clustering in later tests could have resulted from the presence of extreme temperatures at the ends of the tanks.

4.6 RED ABALONE (HALIOTIS RUFESCENS) THERMAL PREFERENCE/AVOIDANCE

According to Morris et al. (1980), the red abalone, (Haliotis rufescens), is common along rocky portions of the California coast in the intertidal and subtidal regions. It is the largest of the abalone species in California. Its geographical distribution extends from Sunset Bay, Oregon to Turtle Bay, Baja California, Mexico. Red abalone support a sport and commercial fishery in California.

Red abalone was designated a representative important species (RIS) for the Diablo Canyon 316(a) demonstration. The literature on red abalone was reviewed in a previous report (PGandE 1979a).

The objective of the thermal preference/avoidance experiments was to provide information on the behavioral response of red abalone to a thermal gradient, specifically, whether it exhibits preference for or avoidance to a particular temperature. The purpose was to provide information about the possible response of the species to the thermal plume, in particular, whether sublethal temperatures would have a major effect on its distribution in Diablo Cove.

4.6.1 METHODS

A summary of the methods employed in the thermal preference/avoidance experiments on red abalone is presented below. A more detailed account of the experimental procedure used in this experiment is presented in APPENDIX B (Procedure 317). The protocols for this experiment are included in APPENDIX C of this report (experiments 17c and 17e-i).

The juvenile specimens of red abalone used in this experiment were originally obtained from a commercial supplier and were held for an extended period in outdoor tanks with ambient flow-through seawater. They were then used in the snail growth experiment and they were considered to be acclimated to the test temperatures at that time. The animals were greater than 6 mm in length.

A total of six runs were made on this species. A summary of the run data is presented in TABLE 4.6-1. While being held in the laboratory prior to experimentation, the animals were fed to excess with Macrocystis and maintained in ambient temperature 4 x 8 ft flow-through tanks. During the holding period, the animals 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 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.

The 3-m gradient apparatus used for the experiment is described in detail in APPENDIX A. A brief description of the equipment is given here for the reader's convenience.

The 3-m gradient tank is designed to produce a horizontal thermal gradient to assess behavioral response of various large motile organisms. The gradient apparatus consists of a 380-cm-long tank with two primary heating and cooling chambers (40 x 60 x 45 cm) at both ends of the experimental chamber (300 x 20 x 45 cm). Eleven pairs of surface and bottom baffles divide the experimental chamber into 12 compartments. These baffles impede thermal convection currents and are capable of being raised or lowered to accommodate varying sized organisms and water depth. Gentle aeration from the bottom of each compartment eliminates any vertical stratification within compartments.

Heating and cooling is accomplished primarily through two stainless steel coils located in the end compartments. Two additional heat exchangers of Teflon-coated stainless steel run along the bottom from the ends to the center of the experimental chamber and serve to produce a more uniform temperature gradient. Nylon netting at the ends and along the bottom of the experimental chamber prevents test organisms from entering the heating and cooling chambers and also provides a flat surface over the bottom baffles. Heat is supplied to two of the coils by a 42-gallon hot water heater. A 12-ton chiller cools a slump box

TABLE 4.6-1

PROTOCOL SUMMARY FOR RED ABALONE
THERMAL PREFERENCE/AVOIDANCE

Run	Sample Size	Acclimation Temperature	Gradient Status (Temperature, C)		
			Initial	First Change	Second Change
17c[a]	30	Ambient	8-29	8-22	----
17e[a]	30	"	Ambient	8-26	13-17
17f[b]	14	"	8-30	13-14	----
17g[b]	30	18	7-31	----	----
17h[b]	30	18	8-37	18	----
17i[b]	27	21	7-37	21	----

[a] Run made with Tegula brunnea and Haliotis cracherodii also in the tank.

[b] Run made with Tegula brunnea, T. funebralis and Haliotis cracherodii also in the tank.

which in turn supplies the coolant to the two cold coils. The heatant and coolant are circulated in a closed system by four pumps which are operated by four YSI temperature controllers. A series of piping and control valves allow adjustment of the gradient and the switching of the hot and cold ends of the gradient between experiments. Flow meters are installed in line to facilitate precise temperature control.

The 12 physical compartments are subdivided into 25 observational compartments, in each of which is centered a thermistor probe. Temperatures are recorded on paper tapes by an Autodata Nine computer at predetermined time intervals.

Visual observations were made to determine the presence of the animals in each compartment of the experimental tank. Simultaneous temperature measurements were taken by the Autodata Nine computer and were later combined with the visual observations on one data sheet. The data were keypunched onto magnetic tape and presented graphically by an automatic plotting package.

Because these experiments represented the first attempts to study the behavioral responses of marine invertebrates to a thermal gradient, there were no standard experimental protocols to follow. From the initial runs, it was determined that the most efficient method of conducting the experiment was to establish the experimental temperature gradient in the experimental tank and to initiate the run by placing the test population in the compartment at the acclimation temperature for the test organisms. The organisms were then allowed to disperse in the tank for a period of hours without changing the temperature gradient. After at least one observation, recording of the position of the animals was done, the gradient was discontinued and the entire tank was maintained at the acclimation temperature of the organisms. Again after a period of hours, a final observation was made to verify that the animals could disperse throughout the tank. To a certain extent, the results presented reflect trials of various protocols used in determining the best approach. Once the experimental protocol was established, groups of animals acclimated to different

temperatures were subjected to testing to determine the effects of acclimation on temperature avoidance.

It was possible to include more than one species simultaneously in the invertebrate thermal avoidance experiments, and this was done to the extent possible to increase efficiency. To be grouped together, species had to be compatible with each other (not prey or predators) and of a size and mobility that precluded physical obstruction of movement in the tank.

4.6.2 RESULTS

A summary of the protocols for the runs on red abalone is presented in TABLE 4.6-1. The data are presented in the graphs shown in FIGURES 4.6-1 to 4.6-6.

4.6.2.1 RUN 17C

The test animals were again introduced into an established thermal gradient in the second run on this species, but the temperature range was extended to 7-30 C (FIGURE 4.6-1). At 16 hours into the run, the animals were distributed in two groups: one at the cold end of the gradient tank and the other near the middle of the tank, not beyond about 20 C. After the 16-hour observation, the gradient range was reduced to 8-22 C and at 40 hours, the animals were distributed all across the tank. A large group was still present at the cold end of the tank and it appears likely that the animals that were dispersed over the tank were those comprising the group near the middle of the tank at 16 hours. This cannot be determined for certain because the individual abalone were not identified as to position.

4.6.2.2 RUN 17E

The animals were introduced into compartment 13 with the tank at about 13 C (no thermal gradient) and by 17.5 hours had dispersed over the entire tank (FIGURE 4.6-2). A gradient was then induced ranging from 8-26 C, and at the 26-hour observation this gradient had not significantly affected their distribution

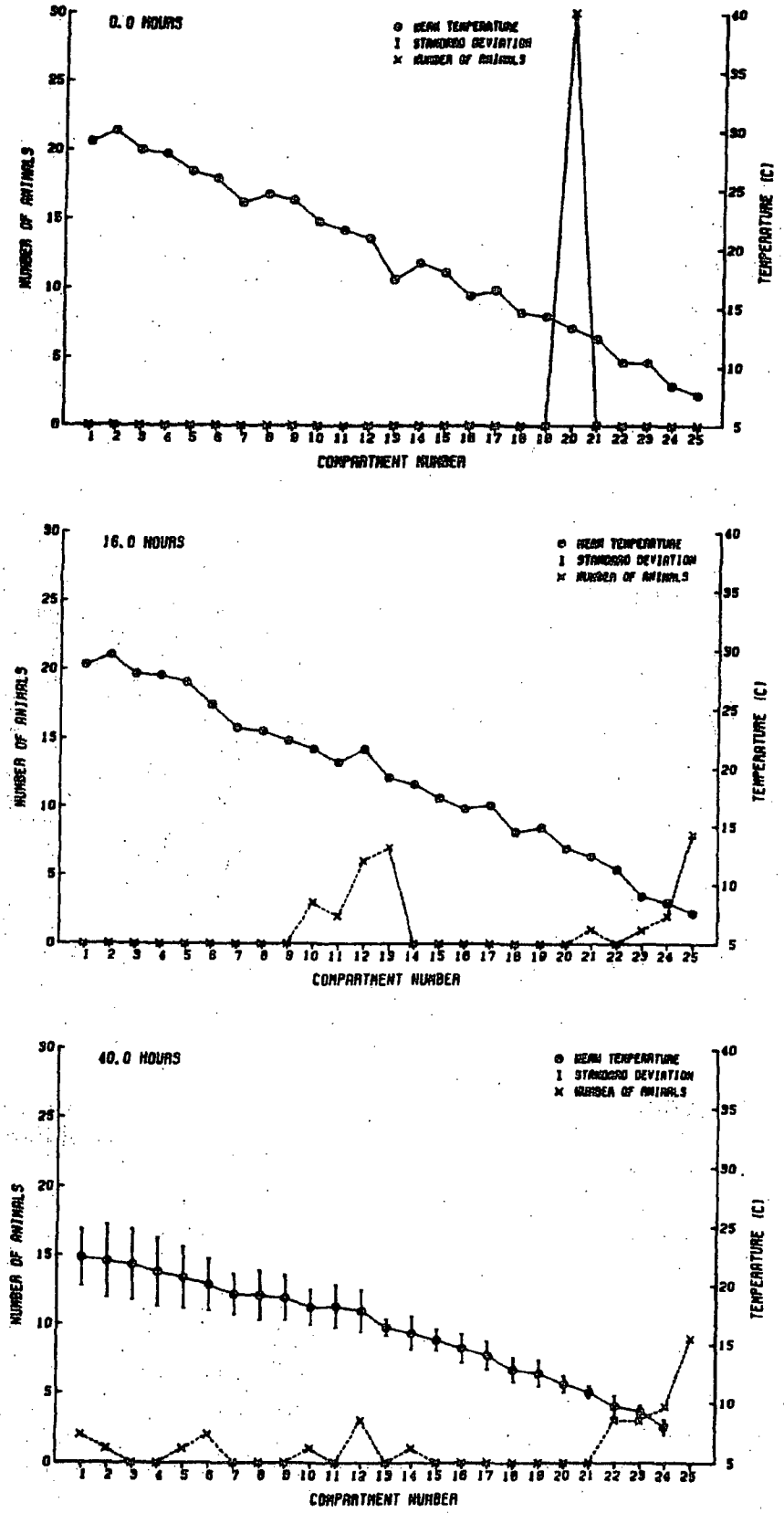


FIGURE 4.6-1

NUMBER OF ANIMALS AND TEMPERATURE VERSUS COMPARTMENT FOR AMBIENT-ACCLIMATED ANIMALS (RUN 17C): RED ABALONE THERMAL PREFERENCE/AVOIDANCE

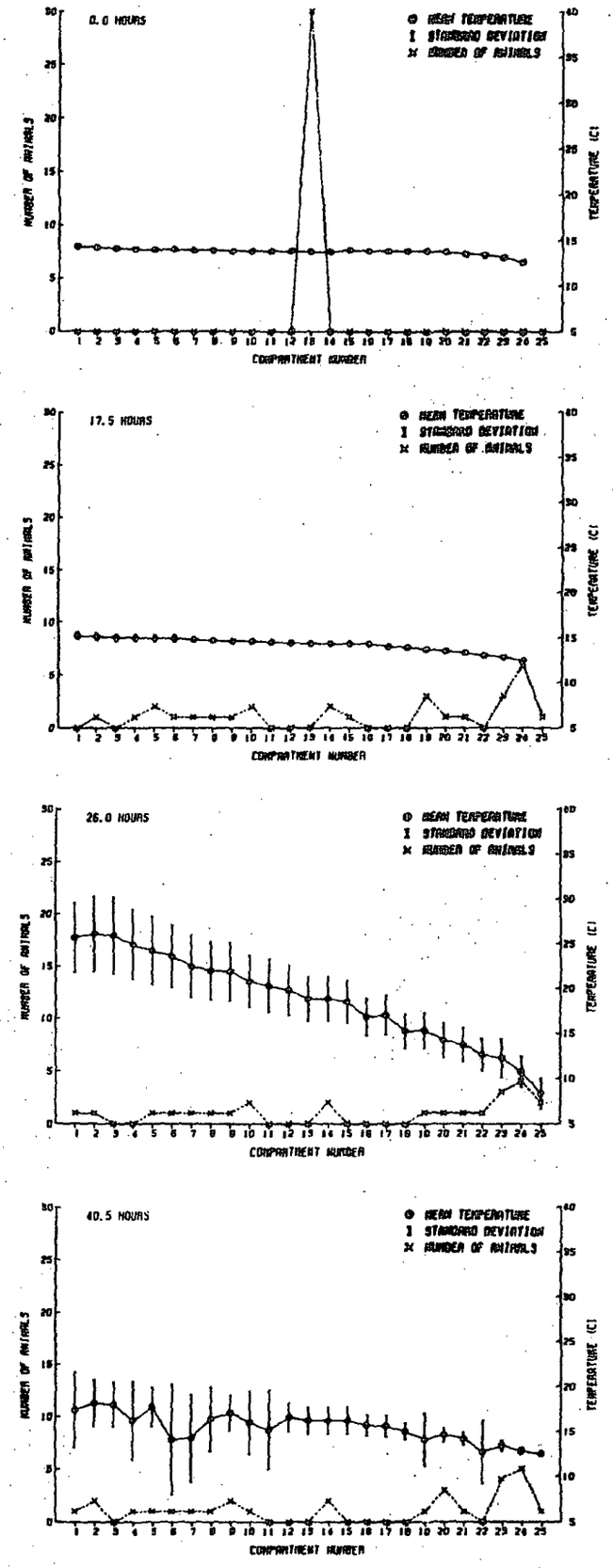


FIGURE 4.6-2

NUMBER OF ANIMALS AND TEMPERATURE VERSUS COMPARTMENT FOR AMBIENT-ACCLIMATED ANIMALS (RUN 17E): RED ABALONE THERMAL PREFERENCE/AVOIDANCE

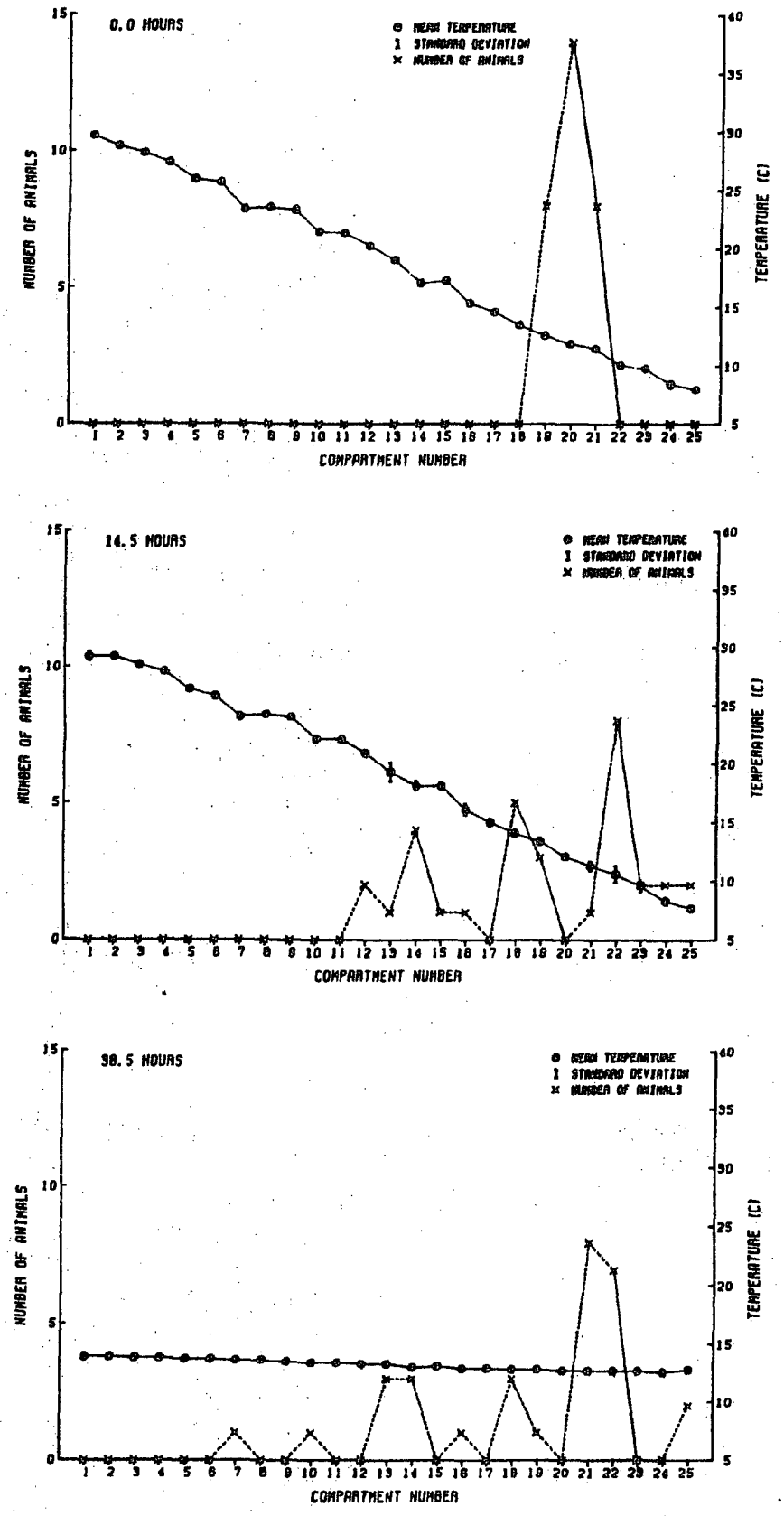
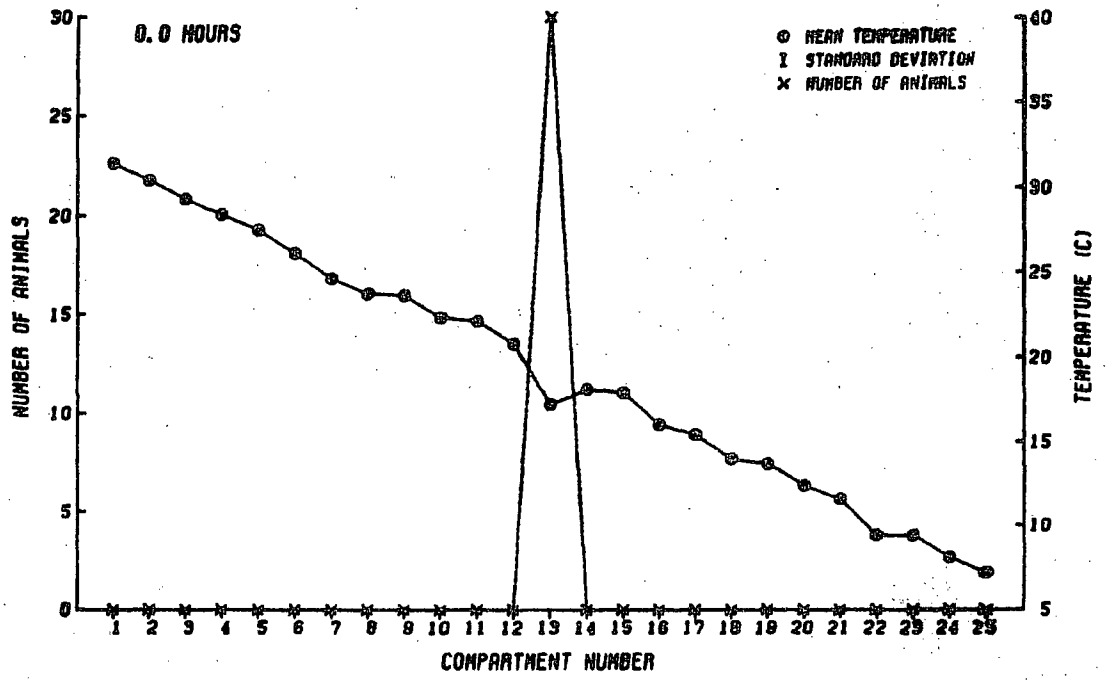


FIGURE 4.6-3

NUMBER OF ANIMALS AND TEMPERATURE VERSUS COMPARTMENT FOR AMBIENT-ACCLIMATED ANIMALS (RUN 17F): RED ABALONE THERMAL PREFERENCE/AVOIDANCE



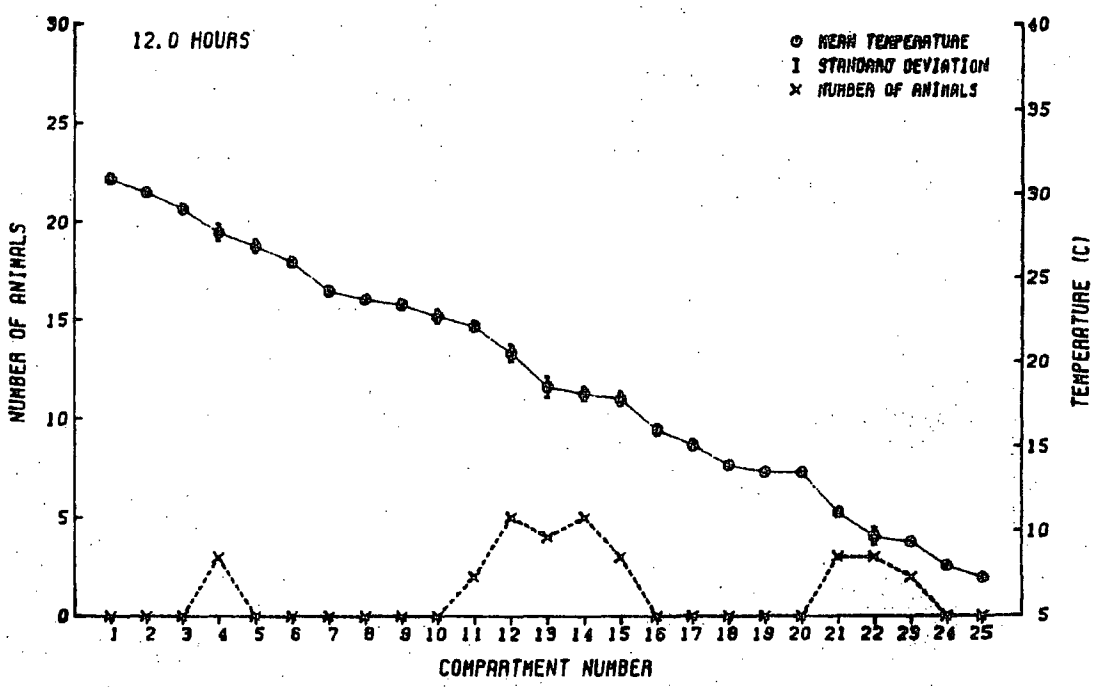


FIGURE 4.6-4

NUMBER OF ANIMALS AND TEMPERATURE VERSUS COMPARTMENT FOR AMBIENT-ACCLIMATED ANIMALS (RUN 17G): RED ABALONE THERMAL PREFERENCE/AVOIDANCE

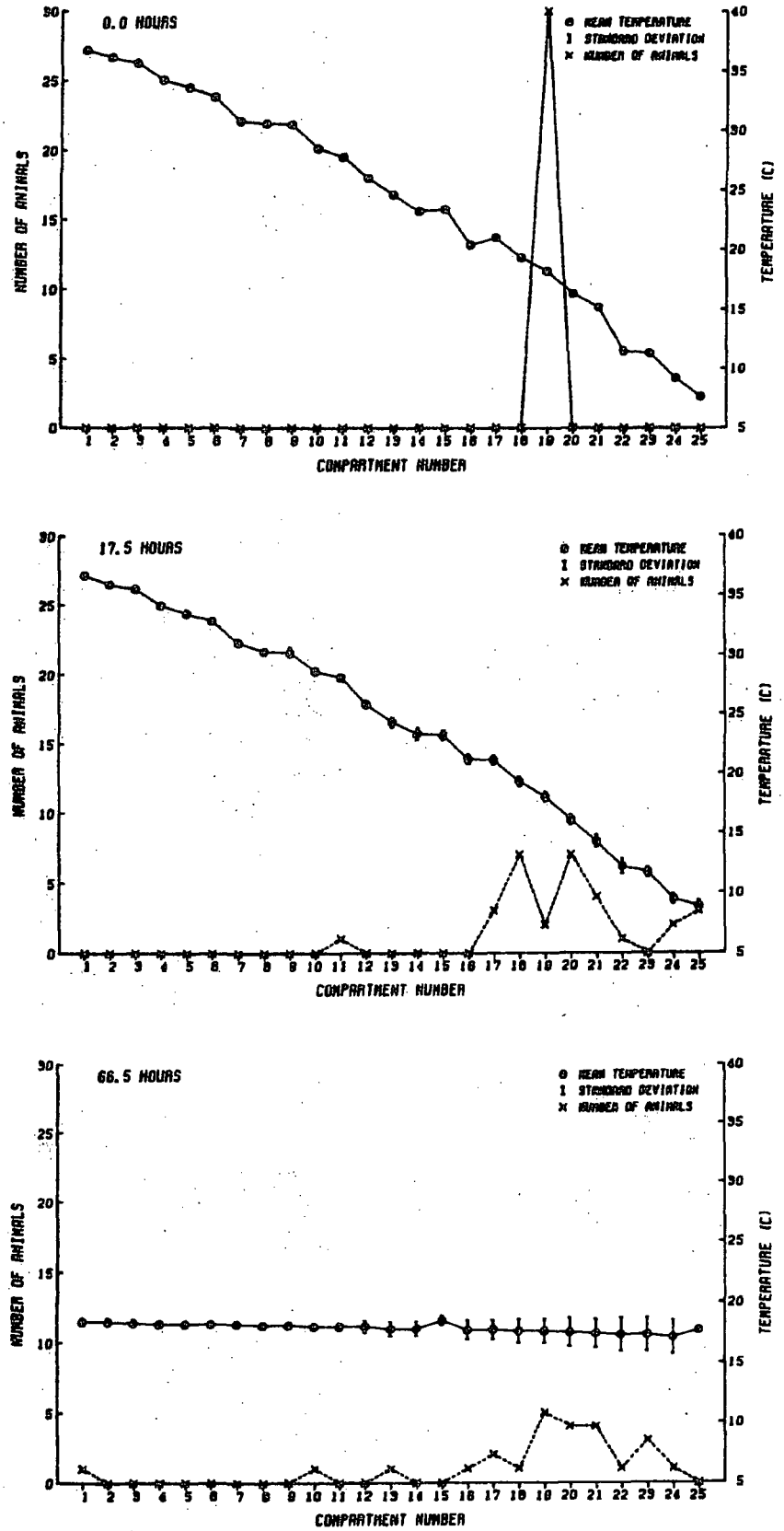


FIGURE 4.6-5

NUMBER OF ANIMALS AND TEMPERATURE VERSUS COMPARTMENT FOR AMBIENT-ACCLIMATED ANIMALS (RUN 17H): RED ABALONE THERMAL PREFERENCE/AVOIDANCE

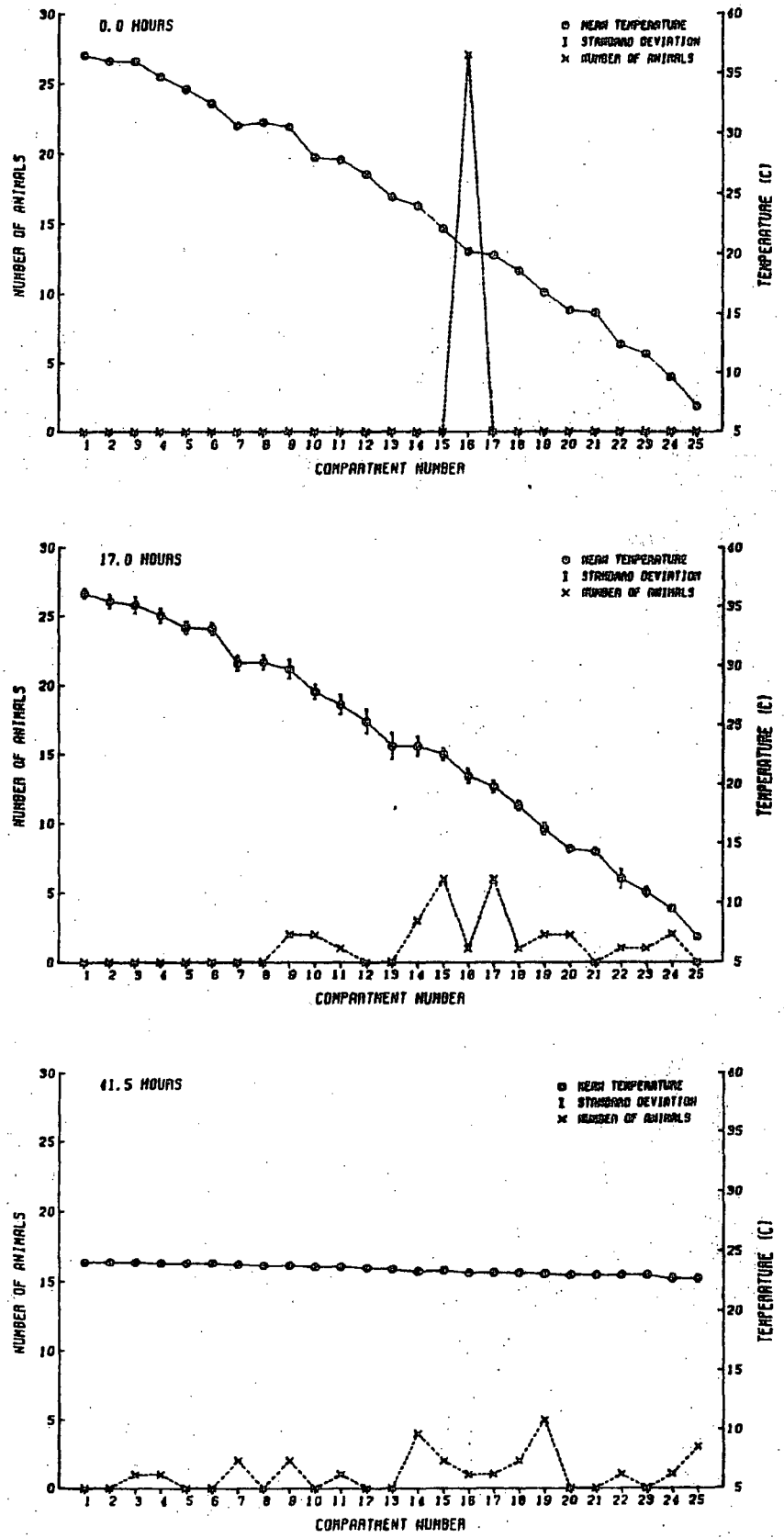


FIGURE 4.6-6

NUMBER OF ANIMALS AND TEMPERATURE VERSUS COMPARTMENT FOR AMBIENT-ACCLIMATED ANIMALS (RUN 171): RED ABALONE THERMAL PREFERENCE/AVOIDANCE

in the tank. The gradient was then reduced to 13-18 C, and at the 40-hour observation the distribution of the animals remained essentially the same.

4.6.2.3 RUN 17F

The animals were introduced into the tank with a gradient already established (8-29 C). By 14.5 hours, the animals had dispersed over the tank up to 20 C (FIGURE 4.6-3). The gradient was subsequently reduced to 13-14 C. By 38.5 hours some abalone had moved into portions of the tank that had been above 20 C at 14.5 hours, although the overall distribution pattern remained the same.

4.6.2.4 RUN 17G

Prior to this run, all animals had been ambient acclimated. In this run, 18 C-acclimated animals from the snail growth experiment were placed at their acclimation temperature within a range of 7-31 C. At 12 hours, they had dispersed up to about 27 C (FIGURE 4.6-4). However, the three animals that had moved to 27 C were not alive at the observation time. Live animals were present at 22 C and below.

4.6.2.5 RUN 17H

This run involved a second test of the 18 C-acclimated animals. A 7-37 C gradient was established prior to placement of the animals in the tank. By 17.5 hours the animals had dispersed over the tank up to 28 C (FIGURE 4.6-5). The animal that had moved to 28 C was found to have died. Live animals were present at 21-22 C and below. The gradient was subsequently reduced to 17-18 C, and by 66.5 hours the animals had dispersed across the tank although the general distribution remained similar to that at 17.5 hours.

4.6.2.6 RUN 171

In this final run on red abalone, 21 C-acclimated animals from the snail growth experiment were introduced into the tank with a 7-36 C gradient. At the 17.5-hour observation, they had dispersed across the tank up to 29 C (FIGURE 4.6-6). However, the animals that had moved above 26 C were not alive at this observation. The gradient was subsequently reduced to 23-24 C, and by 41.5 hours the animals had dispersed across the entire tank.

4.6.3 DISCUSSION

In general, the red abalone tested in this series of experiments exhibited neither an escape nor a preference response to temperature. An avoidance response was generally evident, however, in that most animals did not move into locations where the temperature exceeded a certain (apparently lethal) value. This "avoidance temperature" was positively related to the acclimation temperature of the animals prior to testing (20 C for ambient acclimated, 22 C (71.6 F) for 18 C- (64.4 F-) acclimated and 26 C (78.8 F) for 21 C- (69.8 F-) acclimated animals). A few mortalities were observed when animals moved above the avoidance temperature. There was a tendency of the animals to cluster at the ends of the tank in some runs, but this was not a consistent response.

4.7 ROCK CRAB/ABALONE/SNAIL INTERACTION

Subtle changes in behavioral patterns of marine organisms could result from exposure to differing temperatures within the sublethal range for the species. Of particular importance to species' distributions and abundance patterns and hence community structure, are the interactions of animals in predator/prey relationships. The apparent balance between predator and prey populations in nature results from a complex of population characteristics of one species relative to the other.

The experimental results presented in this and the following section were designed to determine whether temperature significantly affects the predation rate of a predator on two prey species under laboratory conditions. The purpose was to determine whether the power plant thermal plume could affect these behavioral patterns to an extent that might produce changes in the community structure of Diablo Cove.

4.7.1 METHODS

A summary description of the procedure used for the experiment on rock crab/abalone/snail interaction is presented in this section. For a more detailed description, refer to Procedure 326 (APPENDIX B) and protocol for experiment 26b (APPENDIX C).

An initial run of this experiment (26a) was started on 23 April 1980 but was terminated when it was discovered that a number of the prey animals had escaped from their cages. The cages were repaired and reinforced and a second run (26b) was started on 28 April 1980. The experimental set-up entailed placing ten prey animals and one rock crab (Cancer antennarius) adult into each of three compartments in a cage. The cages were 2 x 3 ft x 18 in. overall, constructed of ½-in. PVC pipe tie-wrapped and hot-glued to a piece of 2 x 3 ft flexible thin fiberglass. This framework was enclosed by 1/8 in. black vexar screen attached by tie wraps and hot glue. The cage was divided into three compartments by inserting two 2-ft. sections of vexar inside the cage. The top sides of the

compartments were composed of removable vexar panels attached by velcro fasteners. The compartments held red abalone (Haliotis rufescens) only, black turban snail (Tegula funebralis) only, or equal numbers of both. Three cages were placed into each 4 x 8 ft test tank, providing three replicates of each prey combination, a total of nine compartments per tank. To provide "habitat" for the prey, a single standard 8 x 8 x 16 in. concrete block (previously leached in a flow-through seawater tank at 24 C for at least one week) was placed in each compartment.

The rock crabs were obtained from crab pots set near Diablo Cove several weeks before the experiment. The mean carapace width was 101.4 mm. The red abalone were obtained from a commercial supplier several weeks prior to the experiment and averaged 24-26 mm in shell length. The black turban snails were collected on 11 April 1980 in the intertidal region just south of Diablo Cove and averaged 18-21 mm maximum shell width. All test species were held in the laboratory in ambient temperature flow through tanks. The crabs were periodically fed fish carcasses up to one week before the experiment started (after which they were starved) and the abalone and snails were fed to excess with Macrocystis.

Three temperatures were used during the experiment: 12, 16 and 20 C. Two tanks were programmed for each temperature, giving six replicates of each prey combination. The test tanks were brought to the respective temperatures, the prey animals were placed into the tanks and finally one crab was placed into each compartment. The start time for the experiment was the time at which the predators were placed into the compartments. The numbers of surviving prey were obtained for each compartment 24 hours and 48 hours later. The experiment was terminated at 48 hours.

4.7.2 RESULTS

The statistical summaries of the temperature and prey survival data in the tanks for the duration of the experiment are presented in TABLE 4.7-1. There is a great deal of variability in the survival data, as indicated by the standard

TABLE 4.7-1

SUMMARY TEMPERATURE AND PREY SURVIVAL DATA: CRAB/ABALONE/SNAIL INTERACTION

TEMPERATURE DATA

Target Temperature (C)	Mean Temperature (C)	Standard Deviation
12	12.6	1.4
12	12.1	0.0
16	15.9	0.4
16	16.1	0.4
20	19.7	0.2
20	20.1	0.4

PREY SURVIVAL DATA

Temperature (C)	Period (H)	Abalone	Turban Snail	Abalone/Snail
12	24	2.2(2.6)[a]	5.8(3.2)	1.8(1.5)/2.5(2.3)
12	48	1.2(2.0)	5.4(4.0)	1.2(1.0)/1.8(2.1)
16	24	2.2(2.6)	4.0(4.4)	0.2(0.4)/0.8(1.3)
16	48	0.8(2.0)	4.0(4.4)	0.2(0.4)/0.8(1.3)
20	24	1.7(4.1)	1.8(2.1)	0.3(0.5)/0.8(1.2)
20	48	1.5(3.7)	1.5(1.9)	0.3(0.5)/0.7(0.8)

[a] Mean number animals surviving (six replicates), standard deviation in parentheses.

deviations. There is no statistically significant difference in the numbers of prey surviving; however, there appeared to be a trend toward lower survival of black turban snails with increasing temperature. No similar trend was evident in the abalone data.

4.7.3 DISCUSSION

The predation rate over 48 hours of rock crab on red abalone and black turban snails was not statistically different among 12, 16 and 20 C (54, 61 and 68 F); however, mean values of snail survival showed a trend toward lower survival with increasing temperature. This result indicates that increased temperature may decrease survival of black turban snail but the effect, if it indeed exists, is masked by the variation in survival values.

4.8 SEASTAR/ABALONE/SNAIL INTERACTION

The experimental results presented in this and the preceding section were designed to determine whether temperature significantly affects the predation rate of a predator on two prey species under laboratory conditions. The purpose was to determine whether the power plant thermal plume could affect these behavioral patterns to an extent that might produce changes in the community structure of Diablo Cove.

According to Feder (1963) and Paine (1969) the black turban snail is consumed by the ochre seastar although it may not constitute a majority of its diet, as mussels, (Mytilus spp.) often do. Feder (1963) stated that the snails exhibit escape responses (moving away and climbing out the water) upon introduction of the seastar into water where snails are present. He also observed that the snails became "desensitized" (stopped exhibiting escape responses) after several days. Paine (1969) determined that only the larger older individuals of black turban snails are consumed in the lower intertidal areas of Washington where he sampled. According to Feder (1963) black abalone are rarely eaten by ochre seastars.

4.8.1 METHODS

A summary description of the procedure used for the experiment on seastar/abalone/snail interaction was presented in SECTION 4.7.1. For a more detailed description, refer to Procedure 326 (APPENDIX B) and protocol for experiment 26c (APPENDIX C).

This experiment was started on 2 May 1980. The experimental set-up entailed placing ten prey animals and one ochre seastar (Pisaster ochraceus) adult into each of three compartments in a cage. The compartments held red abalone (Haliotis rufescens) only, black turban snail (Tegula funebralis) only, or equal numbers of both. Three cages were placed into each 4 x 8 ft test tank, providing three replicates of each prey combination, a total of nine compartments per tank. To provide "habitat" for the prey, a single standard 8 x 8 x 16 in. concrete block (previously leached in a flow-through seawater tank at 24 C for at least one week) was placed in each compartment.

The seastars were obtained from pier pilings at Port San Luis several weeks before the experiment. The mean body diameter was from 264-267 mm and the mean body weight was 477-597 g. The red abalone were obtained from a commercial supplier several weeks prior to the experiment and averaged 23-27 mm in shell length. The black turban snails were collected on 11 April 1980 in the intertidal region just south of Diablo Cove and averaged 16-21 mm maximum shell width. All test species were held in the laboratory in ambient temperature flow through tanks. The seastars were periodically fed mussels (Mytilus) up to two weeks before the experiment started (after which they were starved) and the abalone and snails were fed to excess with Macrocystis.

Three temperatures were used during the experiment: 12, 16 and 20 C. Two tanks were programmed for each temperature, giving six replicates of each prey combination. The test tanks were brought to their respective temperatures, the prey animals were placed into the tanks and finally one crab was placed into each compartment. The start time for the experiment was the time at which the predators were placed into the compartments. The numbers of surviving prey were obtained for each compartment 24 hours and 48 hours later. The experiment was terminated at 48 hours.

4.8.2 RESULTS

The statistical summaries of the temperature and prey survival data in the tanks for the duration of the experiment are presented in TABLE 4.8-1. In all treatments, predation was very low or absent and no trends are discernable in the data.

4.8.3 DISCUSSION

The predation rate over 48 hours of ochre seastars on red abalone and black turban snails was very low and exhibited no trends among the 12, 16 and 20 C (54, 61 and 68 F) treatments. This result indicates that temperature has no short-term effect on predation by ochre seastars on red abalone and black turban snails.

TABLE 4.8-1

SUMMARY TEMPERATURE AND PREY SURVIVAL DATA: SEASTAR/ABALONE/SNAIL INTERACTION

TEMPERATURE DATA

Target Temperature (C)	Mean Temperature (C)	Standard Deviation
12	12.1	0.0
12	12.1	0.0
16	16.1	0.0
16	16.1	0.0
20	19.8	0.0
20	20.2	0.0

PREY SURVIVAL DATA

Temperature (C)	Period (H)	Abalone	Turban Snail	Abalone/Snail
12	24	10.0(0.0)[a]	9.7(0.8)	5.0(0.0)/5.0(0.0)
12	48	10.0(0.0)	9.7(0.8)	4.9(0.4)/4.9(0.4)
16	24	9.7(0.5)	9.5(0.5)	5.0(0.0)/4.8(0.4)
16	48	9.7(0.5)	9.3(0.5)	5.0(0.0)/4.0(2.0)
20	24	10.0(0.0)	10.0(0.0)	4.8(0.4)/5.0(0.0)
20	48	10.0(0.0)	10.0(0.0)	4.8(0.4)/5.0(0.0)

[a] Mean number animals surviving (six replicates), standard deviation in parentheses.

4.9 OTHER SPECIES TEMPERATURE PREFERENCE

4.9.1 BLACK SURFPERCH (EMBIOTOCA JACKSONI)

A series of behavioral experiments were designed to examine the thermal preference/avoidance response of selected fish and invertebrate species which are found in the Diablo Cove (discharge) area. This section contains our findings which were reported previously for black surfperch (Embiotoca jacksoni) (PGandE 1979) to illustrate the methods employed in these experiments and the additional experiments completed since the early reports. The following experiments with black surfperch include eleven trials each of ten fish acclimated at 16 C and 12 C.

The experimental apparatus and methods employed in the temperature gradient experiments are briefly summarized below. For additional procedural details, the reader is referred to the laboratory procedure included in APPENDIX B (Procedure 306) and experimental protocols in APPENDIX C (experiments 6a, j, k, m, n, o, q, u, v) of this report. The apparatus employed in these experiments has been described in detail in an early progress report (PGandE 1977, Section 6.2.2.6).

4.9.1.1 METHODS

A sample of 305 black surfperch were collected between 6 June 1978 and 8 August 1978 and a second sample collected from inside Morro Bay, California. The specimens were collected by beach seine, placed in aerated tubs and transported to the Diablo laboratory. The fish were held in ambient (12 C) running seawater tanks and fed a maintenance diet of brine shrimp for an average period of approximately 90 days. During this period of time 96-hour median lethal temperature tests and critical thermal maximum tests were performed on the population.

All of the collected fish were pooled and not kept separate by collection periods. Therefore some proportion of the fish (<25 percent) were held in the laboratory

for as long as 120 days, but the majority of the pooled test specimens were held for less than 60 days before their use in the thermal preference experiments. The effect of holding these fish in the laboratory is unknown; however, some temperature preference change may have occurred due to nutrition and ambient laboratory seawater temperatures which are lower than Morro Bay temperatures. During the holding period (minimum of 48 hours) any injured fish or fish displaying abnormal behavior (schooling, feeding, swimming and equilibrium) were removed from the test populations.

Following the observational holding period, the selected test specimens were transferred to acclimation containers and the water temperatures raised or lowered to the specified acclimation temperatures (12 or 16 C), at the rate of 1 C per day. The fish were held at these acclimation temperatures until acclimation was achieved as measured by no further change in thermal resistance in a series of daily critical thermal maximum (CTM) tests, according to procedure 308. The results of the CTM tests were employed to establish that acclimation was occurring, but full acclimation was probably achieved in the additional 1- to 2-week holding period at acclimation temperatures following the CTM tests and prior to the preference tests.

The acclimated fish were carefully transferred by bucket to the gradient test chamber upon completion of the gradient chamber setup described in APPPENDIX B (Procedure 306). The pretest gradient temperature was established uniformly throughout the gradient at ±0.5 C of the respective 12 or 16 C acclimation temperatures. Photoperiod (lighting timer switch) was set to 14-hour light and 10-hour dark beginning with a lighted condition.

Positions of the fish in the gradient are recorded by three overhead 35 mm cameras with strobe lights that are automatically triggered by an electronic timer. The gradient temperature is measured electronically in each of the 25 gradient chamber compartments by thermistors imbedded in the chamber floor. The compartment temperatures are recorded manually on a YSI 81A recorder and automatically on an Autodata Nine temperature logger at 60-minute

intervals. Digital time displays are positioned in the gradient chamber so that they appear in each photograph along with the compartment numbers.

The temperature range in the gradient is controlled by heating and chilling units with inflows to four heat exchangers, two at the ends and two on the bottom of the gradient chamber. Between runs, the hot and cold water inflows can be reversed to test any end preference effects. The gradient chamber is equipped with air supply airstones in each compartment to provide aeration and vertical circulation to destratify the gradient water temperatures.

The start of an experimental run is initiated by introducing the ten test fish (normally ten individuals depending upon size of organism and behavioral traits) into the gradient at their pre-experimental acclimation temperatures. The fish are left in this condition for a period of 15 to 17 hours. At the end of this time, the temperature controllers are set to -6 C acclimation temperature (ΔT) at the cold end, -3C ΔT, mid-gradient cold end, +3 C ΔT mid-gradient warm end, and +6 C ΔT warm end. The cameras and temperature recording systems are set to 5-minute intervals and operated at this interval for 60 minutes. At the end of this transition period, the recording rate is reset to 15-minute intervals. The gradient is then maintained for 120 minutes with no less than a 10 C difference between the hot and cold ends. After this stable gradient period, the gradients are routinely adjusted over the remaining course of the experiment in order to treat the chamber compartments with a range of temperatures and obviate compartment selections. This observation period continued for a period of not less than 7 hours and up to 24 hours. In this present experiment, the observation period from the establishment of the gradient was continued for a period of 8 hours (less than 24 hours from the introduction of the fish into the gradient chamber).

At the termination of the experiment, the fish were removed from the gradient chamber, anesthetized, weighed and measured and returned to the wild. The photographic films were developed and projected using three side-by-side projectors, and the compartment positions of the fish were recorded on data sheets for each photographic period. The recorded compartment temperatures at the time

of each photograph were also entered on the data sheet and the data entered into the computer.

Mean selected temperatures for each recording interval were computed according to the formula:

$$MST = \frac{\sum X_i t_i}{\sum X_i}$$

where: MST = mean selected temperature, degrees C

X_i = number of fish in i^{th} compartment

t_i = temperature of ith compartment (degrees C)

i = 2 . . . 24 compartment with fish

The fish and temperatures of the end compartments (compartments 1 and 25) were excluded from the calculation to obviate suspected positional effects on selection behavior.

4.9.1.2 RESULTS

Results of the thermal gradient preference tests for black surfperch acclimated at 12 and 16 C are expressed as the MST. The MST values for the 12 and 16 C acclimated fish were summarized by recording periods during the course of the experiment and are shown in FIGURES 4.9.1-1 through 4.9.1-5. The experiment was broken into an initial 14-15-hour period characterized by minimum thermal gradient. In practice a small gradient was present during this period due to the need to heat and chill in order to maintain a stable temperature (acclimation temperature). The largest temperature differences usually occurred in the last two or three warm end compartments. As seen in FIGURE 4.9.1-5, the MST for the 12 and 16 C test groups during this acclimation period were very close to the nearly uniform gradient acclimation temperatures. However, the black surf-

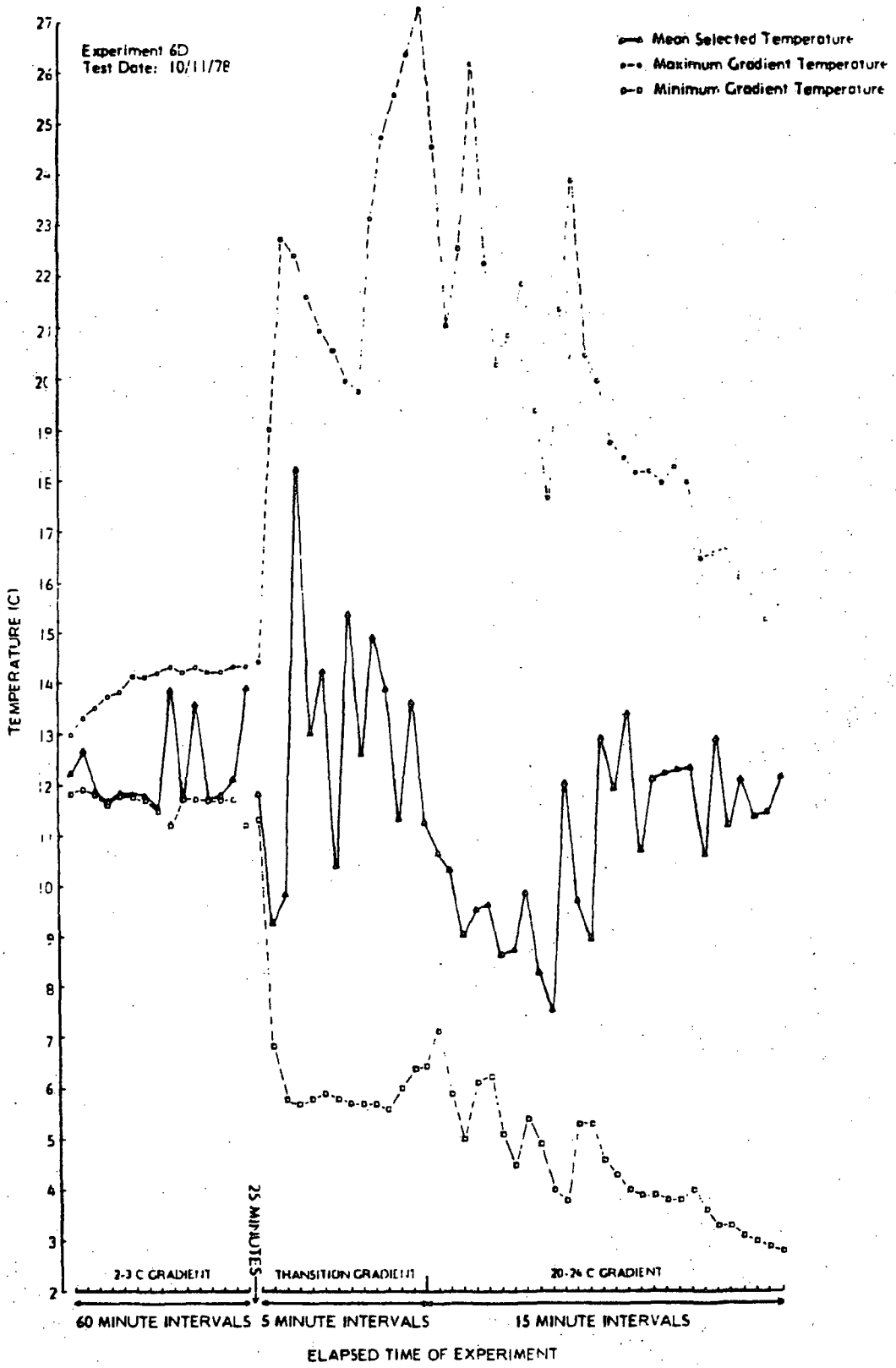
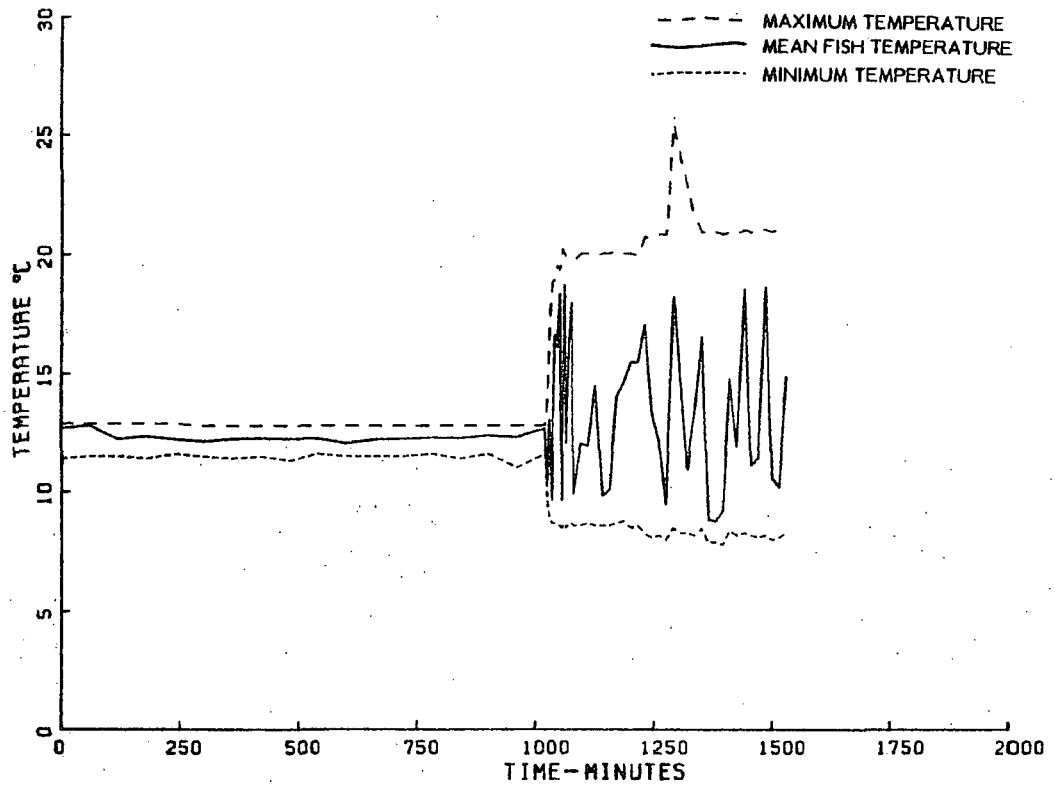
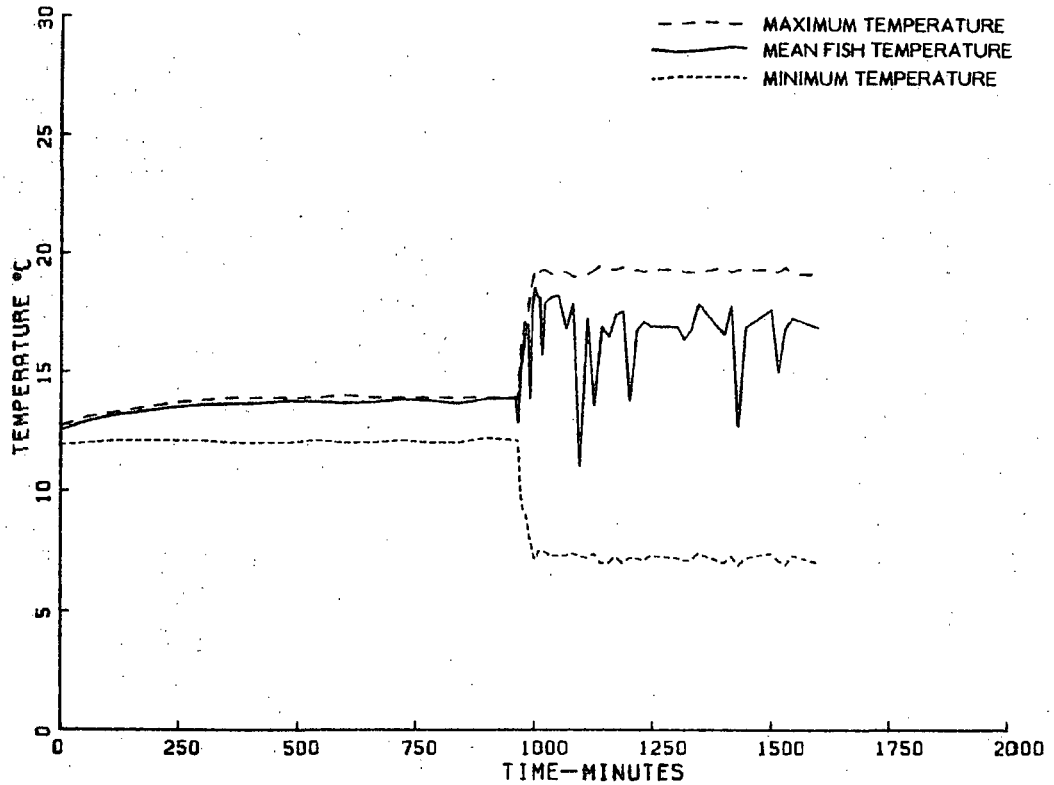


FIGURE 4.9.1-1

THERMAL GRADIENT MEAN SELECTED TEMPERATURE VERSUS TIME OF SUBADULT BLACK SURFPERCH (N = 10) ACCLIMATED AT 12 C (1978)



EXPERIMENT = 6AM



EXPERIMENT = 6AI

FIGURE 4.9.1-2

THERMAL GRADIENT MEAN SELECTED TEMPERATURE
VERSUS TIME OF SUBADULT BLACK SURFPERCH
(N = 10) ACCLIMATED AT 12 C (1979)

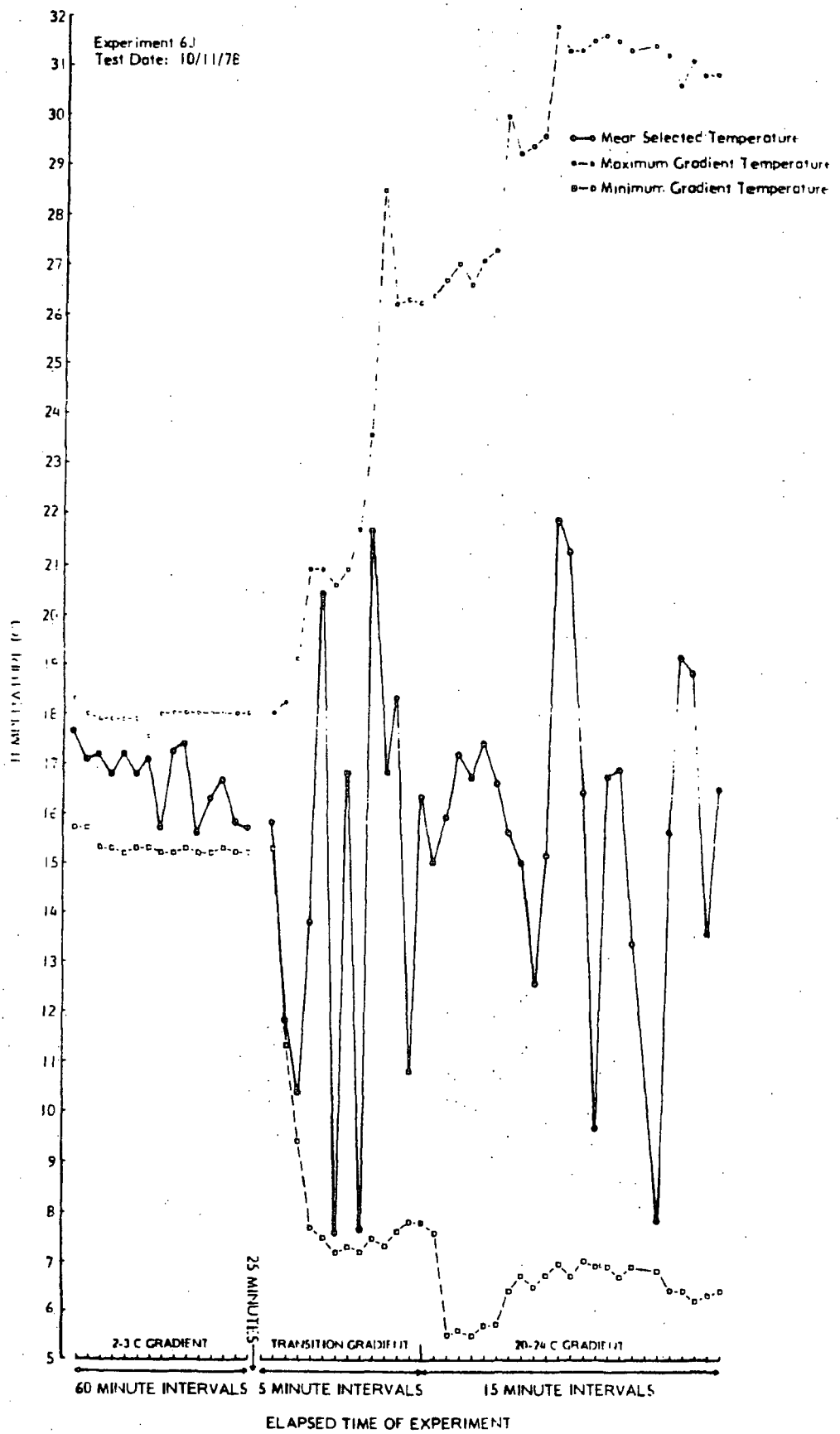
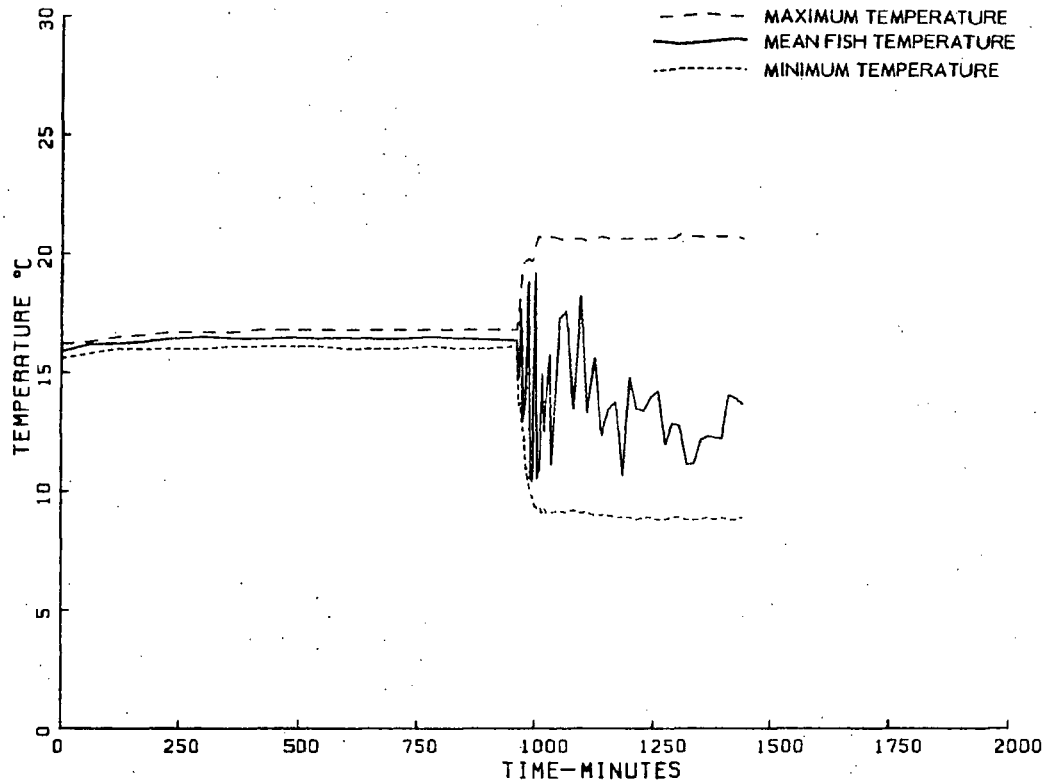
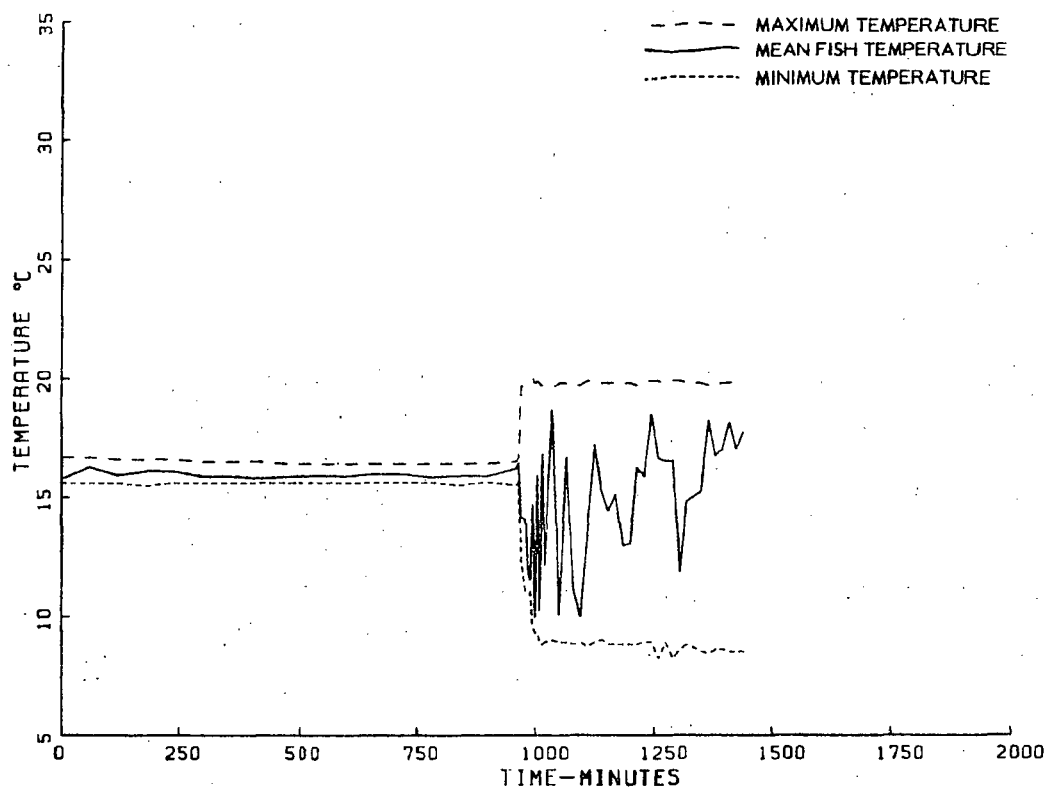


FIGURE 4.9.1-3

THERMAL GRADIENT MEAN SELECTED TEMPERATURE VERSUS TIME OF SUBADULT BLACK SURFPERCH (N = 10) ACCLIMATED AT 16 C (1978)



EXPERIMENT = 6AO



EXPERIMENT = 6AQ

FIGURE 4.9.1-4

THERMAL GRADIENT MEAN SELECTED TEMPERATURE
VERSUS TIME OF SUBADULT BLACK SURFPERCH
(N = 10) ACCLIMATED AT 16 C (1979)

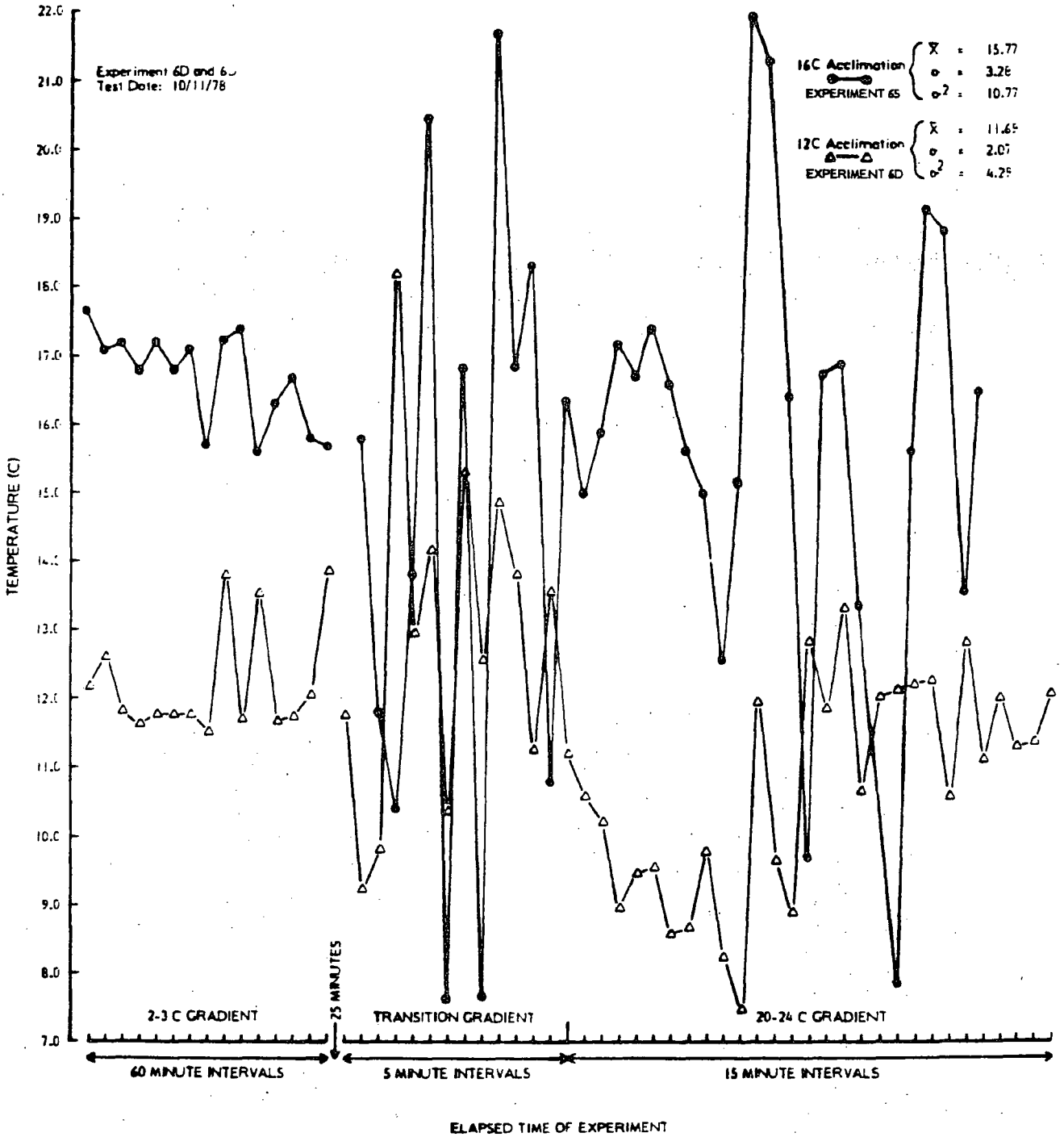


FIGURE 4.9.1-5

BLACK SURFPERCH TEMPERATURE PREFERENCE:
MEAN SELECTED TEMPERATURE VERSUS TIME
FOR 12 AND 16 C ACCLIMATED FISH

perch exhibited a high degree of schooling behavior and infrequent excursions into the warm end compartments registered as high MST values in the 12 C fish test. The 16 C fish during this period of time tended to move from warm temperatures to cooler temperatures.

Following this 14- to 15-hour period of gradient chamber acclimation, an experimental temperature gradient was established by increasing the heating and cooling at opposite ends of the gradient. Observations were made at five-minute intervals for 60 minutes during this transition to a wide range gradient. In most cases, the response of the 12 and 16 C acclimated groups to the introduced gradient was to move towards the cooler temperatures and then to the warmer temperatures. Assuming the initial response represents a form of exploratory behavior in the new temperature regime, the 12 C fish exhibited a reduction in search behavior over this one-hour transition period. The MST values of the 12 C and the 16 C acclimated group MST values ranged widely from the hot to cold temperatures during the transition period. Their search behavior from hot to cold ends continued with little change during the course of the experiment.

To compare the behavioral response of the 12 and 16 C acclimated fish, the MST values shown in FIGURES 4.9.1-1 through 4.9.1-4 were combined in FIGURE 4.9.1-5. Inspection of the two curves illustrates a marked difference in the behavioral response of the two test groups. A much higher degree of variation in the MST values is shown in the 16 C fish than in the 12 C fish ($F = 2.51$, $p = 0.01$). A slight trend towards convergence of the two curves can be observed over the course of the experiment. The species' MST appears to correspond directly to the population's acclimation temperature, based on the two acclimation temperatures studied in this experiment.

The calculated overall MST for the 1978 experiments (6d and 6j) for the 12 C acclimated fish was 11.6 ± 2.0 C and 15.8 ± 3.3 C for the 16 C acclimated fish. This overall value was based on all the observations during the full course of the experiment (12 C fish, $n = 563$; 16 C fish, $n = 502$). In a small number of cases, all ten of the fish in the gradient could not be detected in a photograph;

therefore, a small number of the MST values at an observation period are based on fewer than ten fish.

The calculated overall MST for the 1979 experiments (6ai, ak, am, an, ao, aq, t, u and v) are listed in TABLE 4.9.1-1 for both 12 C- and 16 C-acclimated fish. The MST values range from 12.8 C to 16.6 C and are on the average lower for the 12 C-acclimated fish. No significant differences are evident between the 1978 and 1979 test results.

The frequency of occurrence of 12 and 16 C acclimated fish with respect to temperature is illustrated in FIGURES 4.1.9-6 through 4.1.9-7 for the 1978 experiments 6d and 6j. These figures also show the overall fish frequencies by each of the major periods of the experiment (minimum gradient less than 930 minutes, transition gradient greater than 930 but less than 985 minutes, and maximum gradient above 985 minutes). The overall maximum and minimum MST values for the 12 C acclimated fish were 18.2 and 7.5 C. The maximum and minimum MST values for the 16 C acclimated fish were 21.9 and 7.6 C. Given the wide temperature range in the gradient, it appeared that warm water (16 C acclimated) black surfperch avoid temperatures above 22 C and cool water (acclimated to 12 C) avoid temperatures above 19 C.

The results of the 1979 test were analyzed in a similar manner and are illustrated graphically in a slightly different format as shown in FIGURES 4.9.1-8 and 4.9.1-9. The temperature gradient patterns of fish distribution varied considerably among experiments and produced test results that were nearly mirror-image reversals (as seen in experiments 6aj and 6au). Examining all of the 1979 test results in a summary fashion, it can be stated that the fish preferred temperatures below 20 C (in most experiments avoided temperatures above 20 C) and were variably distributed throughout the gradient without distinct patterns other than the tendency towards the MST.

TABLE 4.9.1-1

MEAN SELECTED TEMPERATURES (MST) OF BLACK SURFPERCH IN THERMAL GRADIENT EXPERIMENTS

Experiment Code Number	Acclimation Temperature (°C)	MST (°C)	Standard Deviation	Number of Observations
6ai	12	16.6	2.2	326
6ak	12	14.8	3.3	397
6am	12	13.5	3.4	368
6an	16	12.8	2.7	252
6ao	16	13.6	2.5	350
6aq	16	15.0	2.8	319
06u	16	13.1	2.8	650
06v	16	16.2	4.3	457

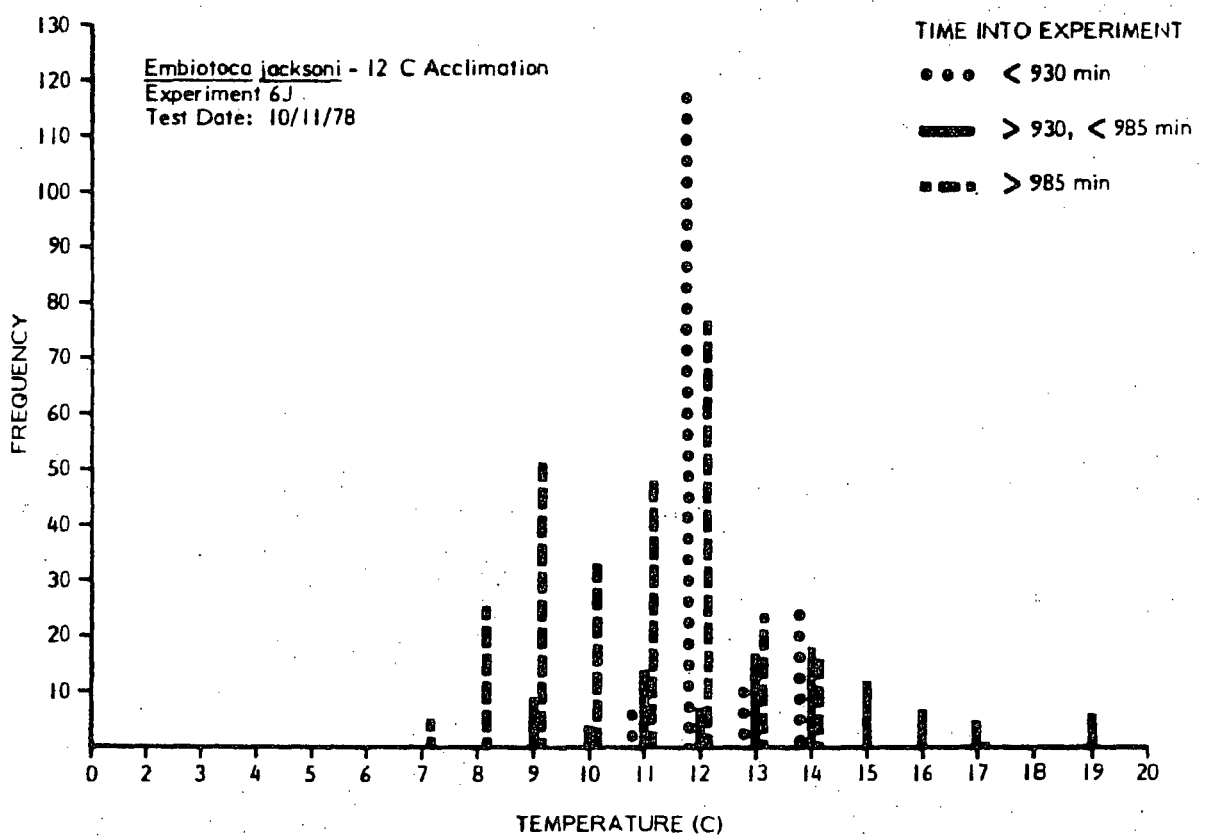


FIGURE 4.9.1-6

BLACK SURFPERCH TEMPERATURE PREFERENCE:
FREQUENCY OF OCCURRENCE OF 12 C ACCLIMATED FISH
VERSUS TEMPERATURE IN THREE PERIODS OF ONE EXPERIMENTAL RUN

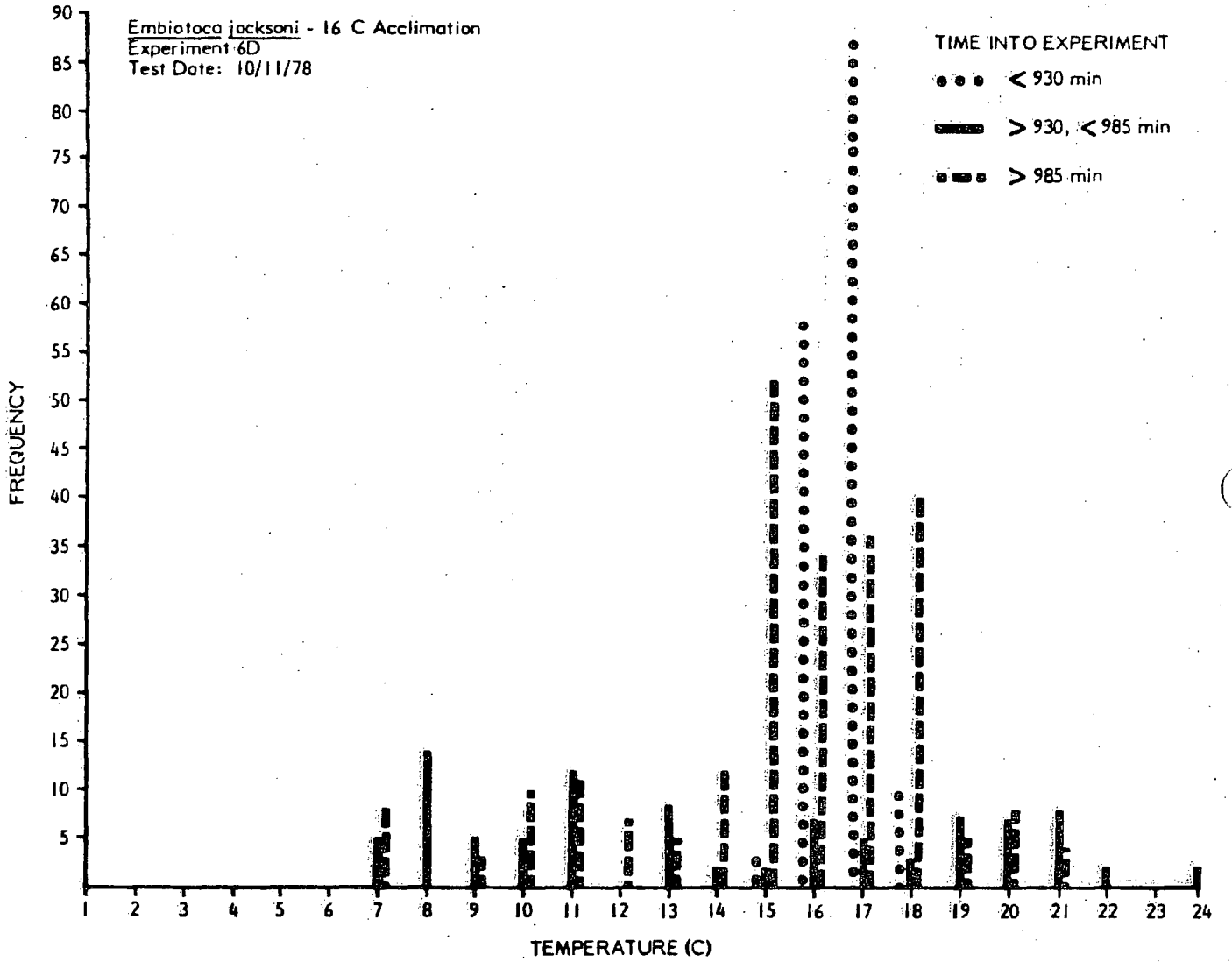


FIGURE 4.9.1-7

BLACK SURFPERCH TEMPERATURE PREFERENCE:
FREQUENCY OF OCCURRENCE OF 16 C ACCLIMATED FISH
VERSUS TEMPERATURE IN THREE PERIODS OF ONE EXPERIMENTAL RUN

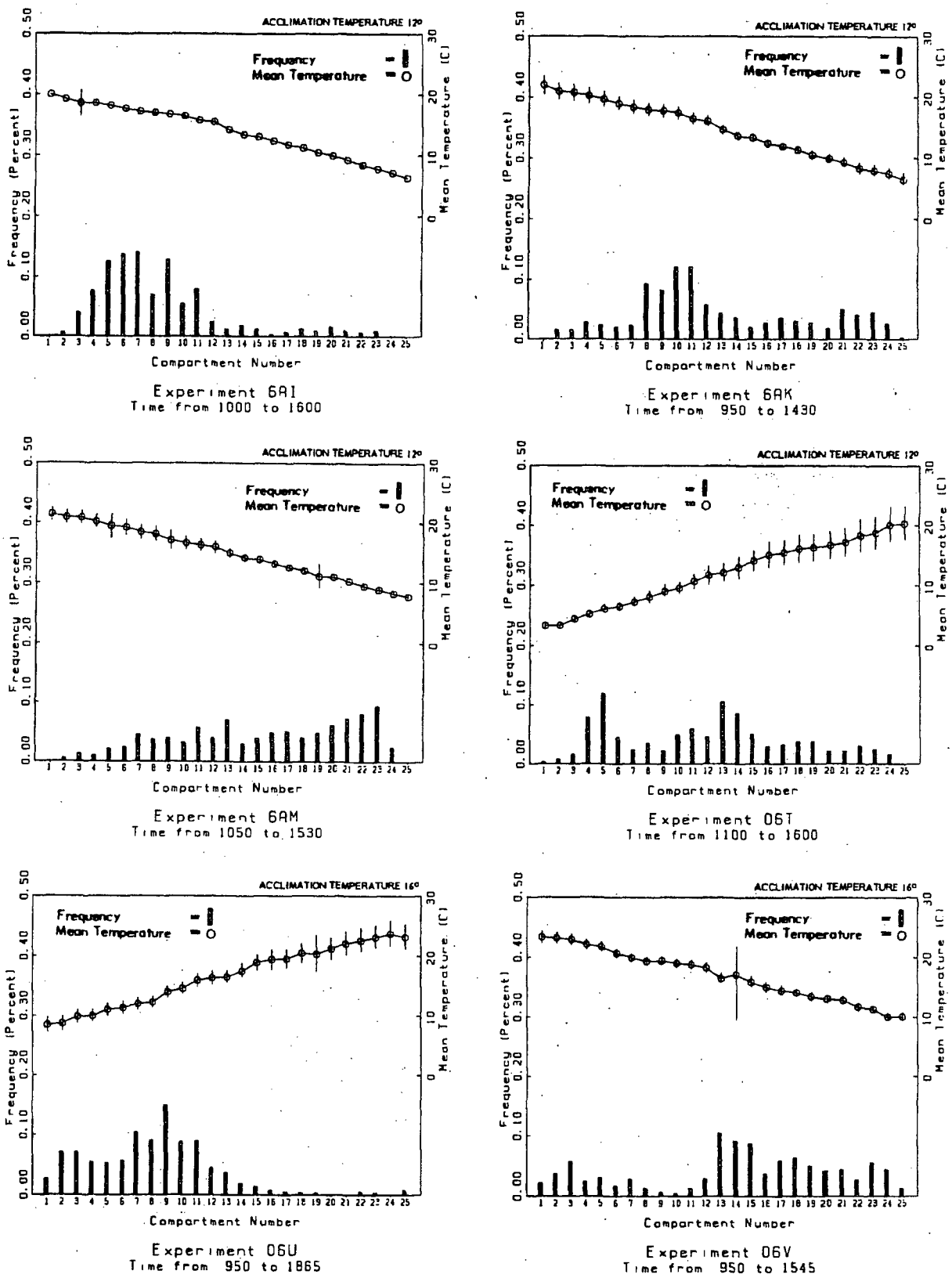
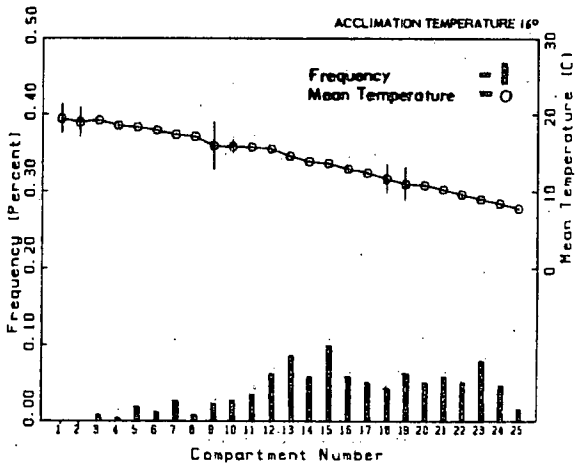
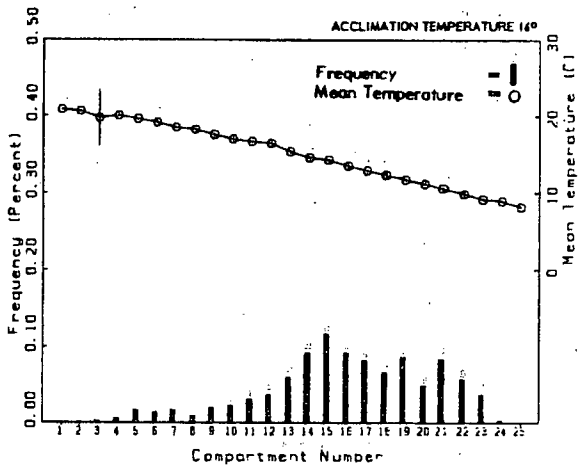


FIGURE 4.9.1-8

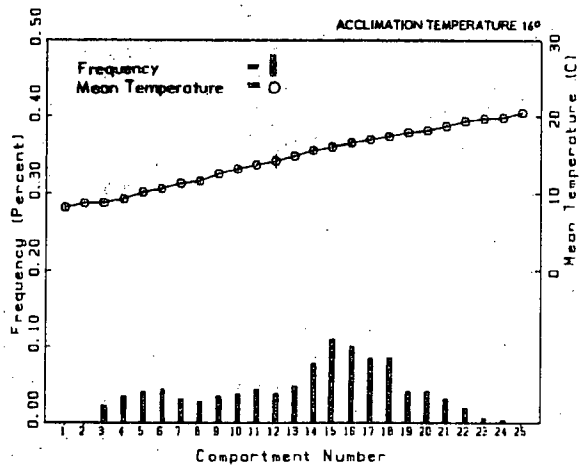
MEAN TEMPERATURE AND PERCENT FREQUENCY DURING OBSERVATION PERIOD (MINUTES) OF SUBADULT BLACK SURFPERCH BY THERMAL GRADIENT COMPARTMENT



Experiment 6AN
Time from 1050 to 1455



Experiment 6AO
Time from 1000 to 1440



Experiment 6AQ
Time from 1000 to 1440

FIGURE 4.9.1-9

MEAN TEMPERATURE AND PERCENT FREQUENCY DURING OBSERVATION PERIOD (MINUTES) OF SUBADULT BLACK SURFPERCH BY THERMAL GRADIENT COMPARTMENT

4.9.1.3 DISCUSSION

These results of the laboratory temperature gradient behavior tests will enable environmental assessments of the thermal discharge plume on the distribution of animal species in Diablo Cove. Many fish and some invertebrate species have been shown to respond significantly in their habitat selection to water temperatures. In this respect, there is a potential for some species to be thermally excluded from the discharge region (loss of habitat) or attracted to the discharge region.

In addition, these results of the temprature gradient behavior experiments were undertaken to gain information on the species' optimum temperature for growth. Based on experiments with freshwater fish species, it has been shown that there are examples of a direct relationship between preferred temperature and optimum temperature for growth. However, the relationship has been little researched in marine species.

The results of the black surfperch temperature gradient behavior experiments have reasonably established a region of both upper and lower temperature avoidance depending upon the thermal history of the fish. When integrated with measured or predicted thermal plume characteristics, an accurate accounting of excluded habitat can be assessed for a variety of power plant operating conditions.

The use of black surfperch temperature preference results to estimate the optimum temperature for growth does not seem promising. Based on our findings we would predict that the optimum temperature for growth would be 15.8 ± 3.3 C (60.4 ± 5.9 F) for 16 C (61 F) acclimated fish and 11.6 ± 2.0 C (52.9 ± 3.6 F) for 12 C (54 F) acclimated fish. From our laboratory findings on 60 d measured growth in black surfperch (preceding SECTION 3.10.1), the optimum temperature for growth is at or near 18 C (64 F). Though the true optimum temperature for growth may be an intermediate temperature which was not tested between 16 and 18 C (61 and 64 F), the lack of correspondence between the preferred temperature and optimum growth temperature and the

wide variance in the preferred temperature MST would seem to preclude an accurate inference of optimum growth temperature from our preferred temperature findings. However, it is possible that further analysis of our studies of other species may reveal closer relationships between preferred temperature and optimum growth temperature.

4.9.2 ASTRAEA GIBBEROSA THERMAL PREFERENCE/AVOIDANCE

According to Morris et al. (1980), Astraea gibberosa, the red turban snail, occurs usually in the subtidal region on the rocky California coast down to 80 m depth. Its geographical distribution extends from the Queen Charlotte Islands, British Columbia, Canada to Bahia Magdalena, Baja California, Mexico. It reaches a maximum shell diameter of 50 mm in California.

Although A. gibberosa was not designated one of the representative important species for the Diablo Canyon 316(a) demonstration, it is of common occurrence in the Diablo Canyon area and was included in the temperature preference/avoidance experiments because time and equipment were available.

The objective of the thermal preference/avoidance experiments was to provide information on the behavioral response of A. gibberosa to a thermal gradient, specifically, whether it exhibits preference for or avoidance to a particular temperature. The purpose was to provide information about the possible response of the species to the thermal plume, in particular, whether sublethal temperatures would have a major effect on its distribution in Diablo Cove.

4.9.2.1 METHODS

A summary of the methods employed in the thermal preference/avoidance experiments on A. gibberosa was presented in SECTION 4.5.1 of this report. A more detailed account of the experiment procedure used in this experiment is presented in APPENDIX B (Procedure 317). The protocol for this experiment is included in APPENDIX C (experiment 17k).

Adult specimens of A. gibberosa, between 20-40 mm diameter, were used in these runs. They were collected during early 1979 from the vicinity of Diablo Cove. While being held in the laboratory prior to experimentation, the animals were maintained in ambient temperature 4x8 ft flow-through tanks. During the holding period, the animals 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 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.

The 3-m gradient apparatus used for the experiment is described in APPENDIX A. The procedure used to conduct the experiment was to establish the temperature gradient in the experimental tank and to initiate the run by placing the test population in the compartment at ambient temperature. The organisms were then allowed to disperse in the tank for a period of hours without changing the temperature gradient. After at least one observation recording the position of the animals was made, the gradient was discontinued and the entire tank was maintained at ambient temperature. Again after a period of hours, a final observation was made to verify that the animals could disperse throughout the tank.

4.9.2.2 RESULTS

Thirty-five specimens were placed in the gradient tank at 12 C, the approximate ambient temperature at the time of testing. By 15 hours, the animals had dispersed across the tank up to 24 C (FIGURE 4.9.2-1). The gradient was then removed so that the entire tank was at the ambient temperature and by 39 hours, the animals had dispersed across the entire tank. There appeared to be a tendency of the animals to cluster in the end compartments at the 39 hour observation.

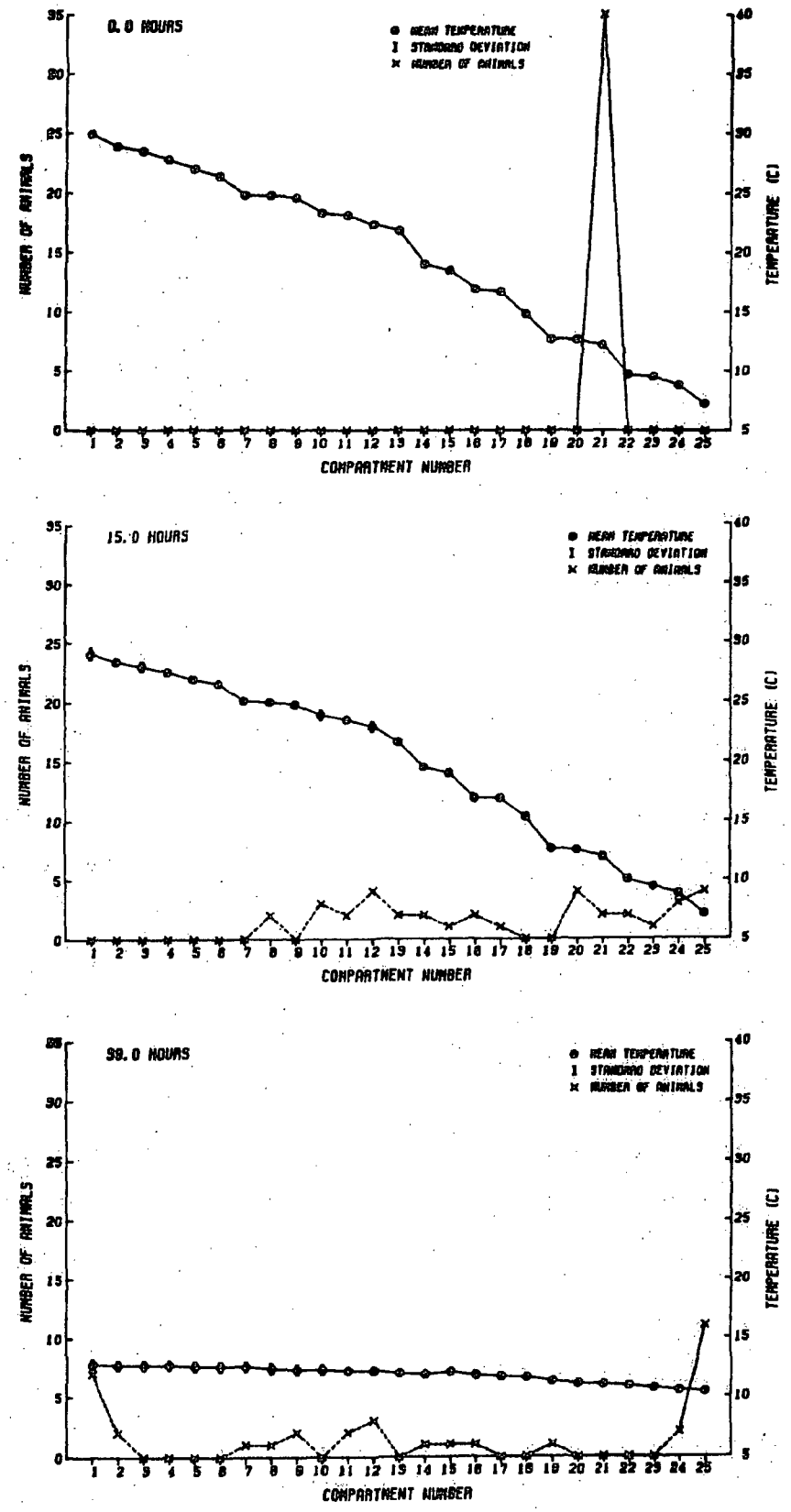


FIGURE 4.9.2-1

NUMBER OF ANIMALS AND TEMPERATURE VERSUS COMPARTMENT:
ASTRAEA GIBBEROSA THERMAL PREFERENCE/AVOIDANCE

4.9.2.3 DISCUSSION

In general, the Astraea gibberosa tested in this experiment did not exhibit a preference response to temperature. An avoidance response was evident, however, in that the animals did not move into compartments where the temperature exceeded a certain value. This "avoidance temperature" was approximately 24 C (75 F).

4.9.3 LEPTASTERIAS HEXACTIS THERMAL PREFERENCE/AVOIDANCE

According to Morris et al. (1980), the seastar Leptasterias hexactis is common on the exposed rocky California coast in the middle intertidal region. Its geographical distribution extends from the Strait of Juan de Fuca, Washington to Santa Catalina Island, Channel Islands, California. It reaches an arm radius of 5.2 cm.

Although L. hexactis was not designated one of the representative important species for the Diablo Canyon 316(a) demonstration, it is of common occurrence in the Diablo Canyon area and was included in the temperature preference/avoidance experiments because time and equipment were available.

The objective of the thermal preference/avoidance experiments was to provide information on the behavioral response of L. hexactis to a thermal gradient, specifically, whether it exhibits preference for or avoidance to a particular temperature. The purpose was to provide information about the possible response of the species to the thermal plume, in particular, whether sublethal temperatures would have a major effect on its distribution in Diablo Cove.

4.9.3.1 METHODS

A summary of the methods employed in the thermal preference/avoidance experiments on L. hexactis was presented in SECTION 4.5.1 of this report. A more detailed account of the experiment procedure used in this experiment is presented in APPENDIX B (Procedure 317). The protocols for this experiment are included in APPENDIX C of this report (experiments 17o, 17z and 17aa).

Specimens of L. hexactis ranging from 20-70 mm were used in these runs. They were collected during early 1979 from the vicinity of Diablo Cove.

A total of three runs was made on this species. A summary of the run data is presented in TABLE 4.9.3-1. While being held in the laboratory prior to experimentation, the animals were fed to excess with Macrocystis and maintained in ambient temperature 4x8 ft flow-through tanks. During the holding period, the animals 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 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.

The 3-m gradient apparatus used for the experiment is described in APPENDIX A.

The procedure used to conduct the experiment involved establishing the experimental temperature gradient in the experimental tank and initiating the run by placing the test population in the compartment at ambient temperature. The organisms were then allowed to disperse in the tank for a period of hours without changing the temperature gradient. After at least one observation recording the position of the animals was made, the gradient was discontinued and the entire tank was maintained at ambient temperature. The final observation was made to verify that the animals could disperse throughout the tank.

4.9.3.2 RESULTS

A summary of the protocols for the runs on L. hexactis is presented in TABLE 4.9.3-1. The data are presented in the graphs shown in FIGURES 4.9.3-1 to 4.9.3-3.

TABLE 4.9.3-1

PROTOCOL SUMMARY FOR LEPTASTERIAS HEXACTIS THERMAL PREFERANCE/AVOIDANCE

Run	Sample Size	Acclimation Temperature	Gradient Status (Temperature, C)		
			Initial	First Change	Second Change
17o	30	Ambient	8-30	12	--
17z	30	Ambient	13	7-29	--
17aa	30	Ambient	7-30	13	--

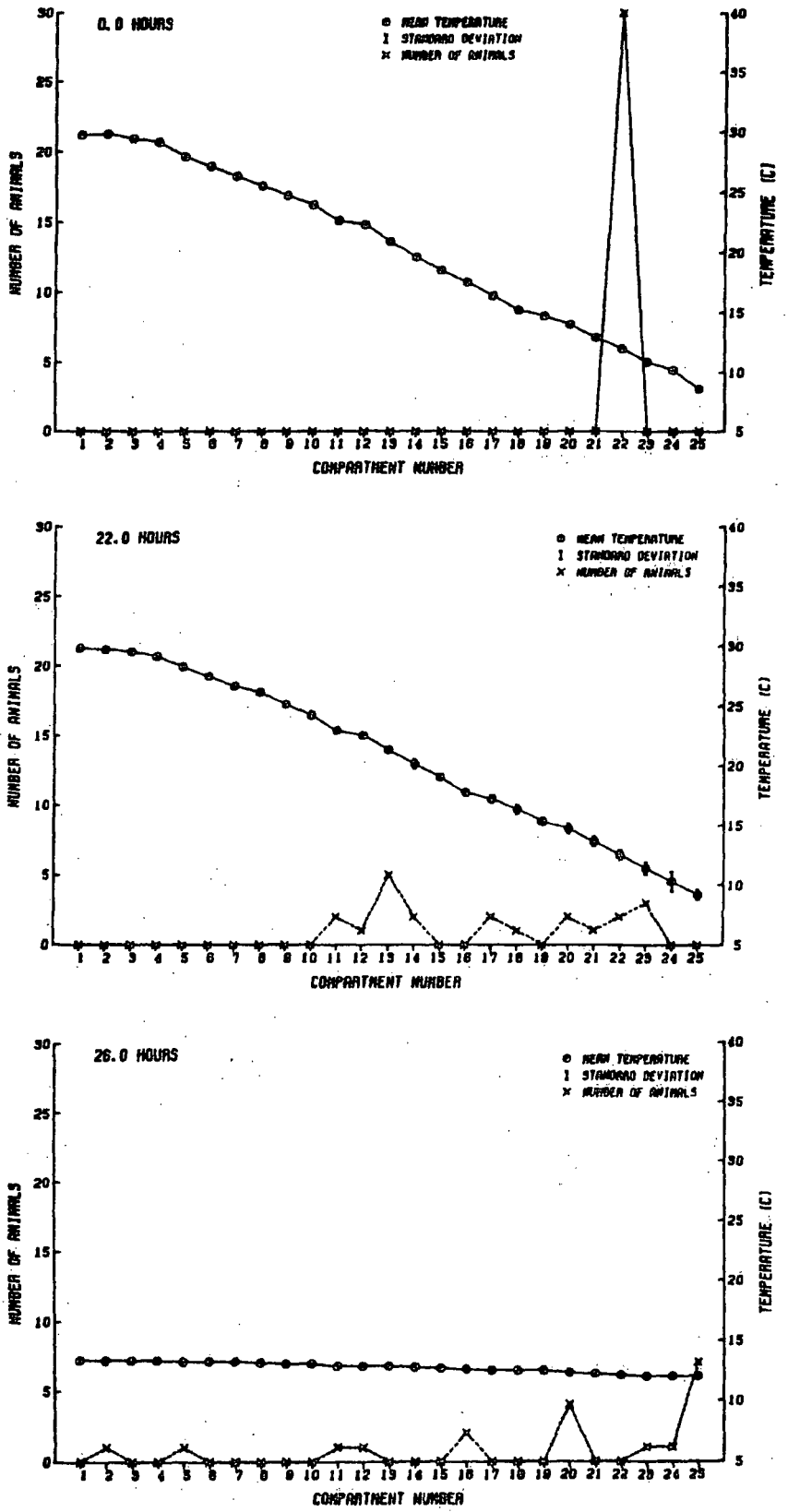


FIGURE 4.9.3-1

NUMBER OF ANIMALS AND TEMPERATURE VERSUS COMPARTMENT:
LEPTASTERIAS HEXACTIS THERMAL PREFERENCE/AVOIDANCE
RUN 170

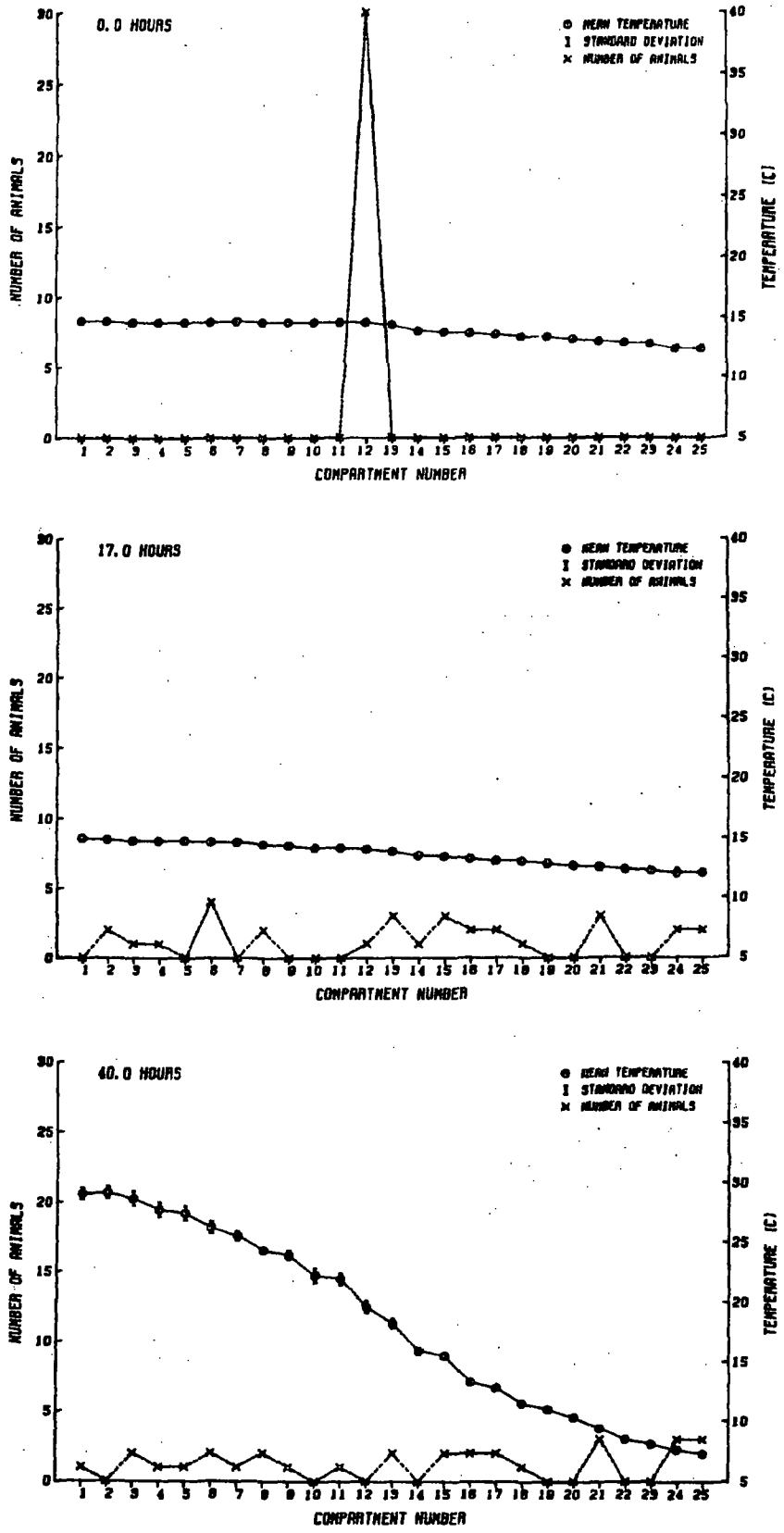


FIGURE 4.9.3-2

NUMBER OF ANIMALS AND TEMPERATURE VERSUS COMPARTMENT:
LEPTASTERIAS HEXACTIS THERMAL PREFERENCE/AVOIDANCE
RUN 17Z

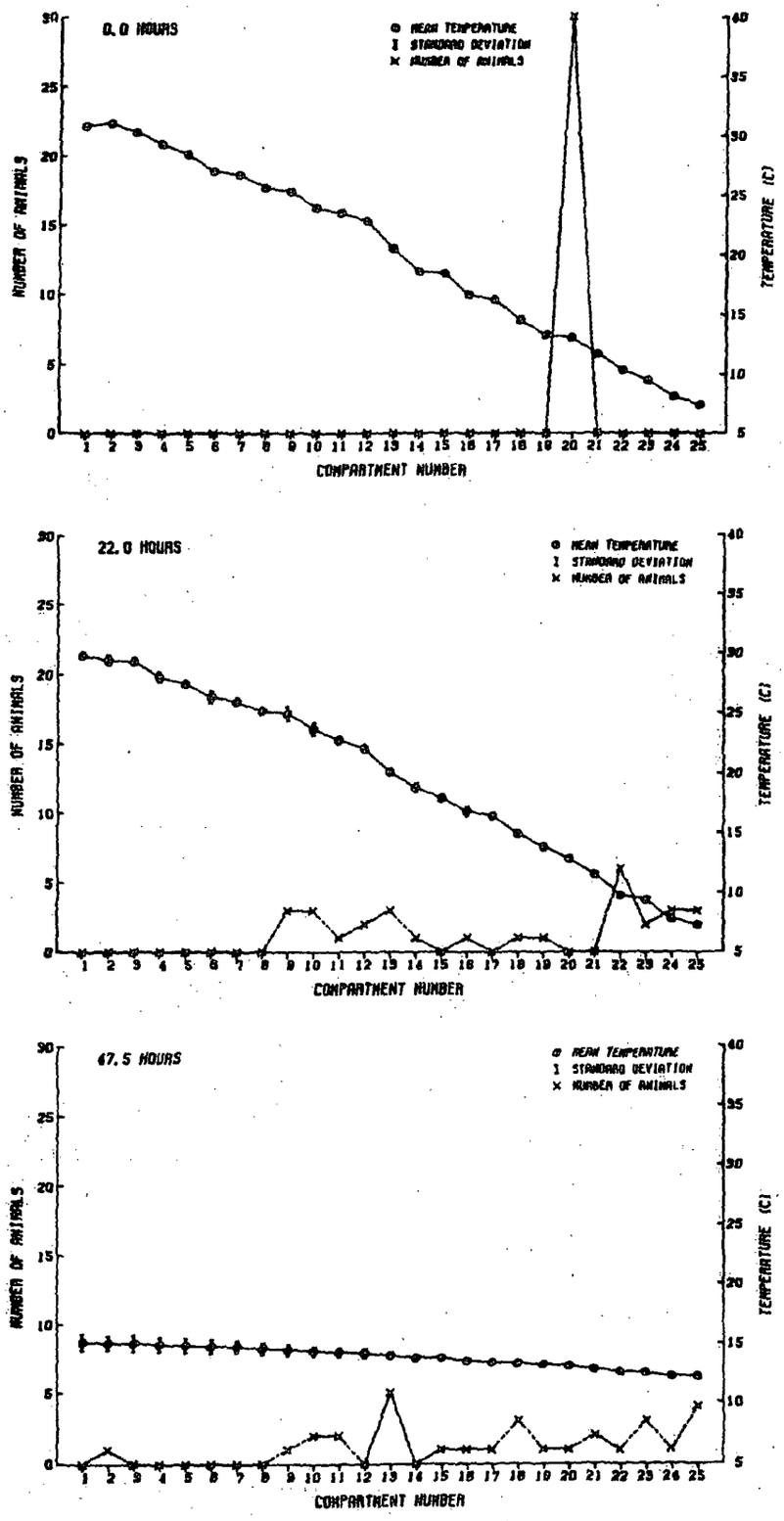


FIGURE 4.9.3-3

NUMBER OF ANIMALS AND TEMPERATURE VERSUS COMPARTMENT:
LEPTASTERIAS HEXACTIS THERMAL PREFERENCE/AVOIDANCE
RUN 17AA

Run 17o

In the first run on this species, 30 animals were placed into the gradient tank in a compartment approximating ambient seawater temperature. The tank had an established gradient of 8-28 C. By 26 hours into the experiment, the animals had dispersed over the tank up to 23 C (FIGURE 4.9.3-1). The gradient was subsequently removed so that all compartments were at about 12 C and by 26 hours, the animals had dispersed across the tank.

Run 17z

In the second run, the test animals were introduced into the tank at ambient temperature without a thermal gradient (FIGURE 4.9.3-2). By 17 hours, they had dispersed over the entire tank. Subsequently, a gradient of 7-28 C was established but the animals did not change their distribution by 40 hours.

Run 17aa

In the final run on L. hexactis, 30 animals were introduced into the tank at their acclimation temperature, with a gradient across the tank of 7-30 C. The animals had become dispersed up to 25 C by 22 hours (FIGURE 4.9.3-3). The gradient was subsequently removed so that all compartments were at approximately 12 C and the animals became dispersed across the entire tank.

4.9.3.3 DISCUSSION

The Leptasterias hexactis tested in this series of experiments consistently exhibited an avoidance response in that the animals did not move into locations where the temperature exceeded 24-25 C (75-77 F). However, this species exhibited neither a preference nor an escape response to temperature. When subjected to temperatures above those established to be avoided, the animals did not move into compartments at cooler temperatures.

4.9.4 PAGURUS GRANOSIMANUS THERMAL PREFERENCE/AVOIDANCE

According to Morris et al. (1980), Pagurus granosimanus, a hermit crab, is common in tidepools of the lower and middle intertidal zones and subtidally to 15 m or more on gravel substrate and kelp holdfasts along the protected rocky California coast. Its geographical distribution extends from Unalaska Island, Alaska to Bahia de Todos Santos, Baja California, Mexico. It reaches a maximum carapace length of 19 mm.

Although P. granosimanus was not designated one of the representative important species for the Diablo Canyon 316(a) demonstration, it is of common occurrence in the Diablo Canyon area and was included in the temperature preference/avoidance experiments because time and equipment were available.

The objective of the thermal preference/avoidance experiments was to provide information on the behavioral response of P. granosimanus to a thermal gradient, specifically, whether it exhibits preference for or avoidance to a particular temperature. The purpose was to provide information about the possible response of the species to the thermal plume, in particular, whether sublethal temperatures would have a major effect on its distribution in Diablo Cove.

4.9.4.1 METHODS

A summary of the methods employed in the thermal preference/avoidance experiments on P. granosimanus was presented in SECTION 4.5.1 of this report. A more detailed account of the experiment procedure used in this experiment is presented in APPENDIX B (Procedure 317). The protocols for this experiment are included in APPENDIX C of this report (experiments 17m and 17w).

Adult specimens of P. granosimanus, with shell diameters ranging from 15-25 mm, were used in these runs. They were collected during early 1979 from the vicinity of Diablo Cove.

While being held in the laboratory prior to experimentation, the animals were maintained in ambient temperature 4x8 ft flow-through tanks. During the holding period, the animals 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 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.

The 3-m gradient apparatus used for the experiment is described in APPENDIX A. The procedure used to conduct this experiment involved establishing the experimental temperature gradient in the experimental tank and initiating the run by placing the test population in the compartment at the acclimation temperature for the test organisms. The organisms were then allowed to disperse in the tank for a period of hours without changing the temperature gradient.

After at least one observation recording the position of the animals was made, the gradient was discontinued and the entire tank was maintained at the acclimation temperature of the organisms. Again after a period of hours, a final observation was made to verify that the animals could disperse throughout the tank.

4.9.4.2 RESULTS

In the first run conducted on this species, 25 animals were introduced into the gradient tank in a compartment approximating ambient seawater temperature. The gradient ranged from 8-30 C. By 15.5 hours into the experiment, the animals showed a strong tendency to cluster in the cool end compartment, although a few individuals were in compartments up to 20 C (FIGURE 4.9.4-1). The gradient was subsequently removed so that all of the compartments were at 12 C. By 22 hours the animals had redistributed toward the warm end of the

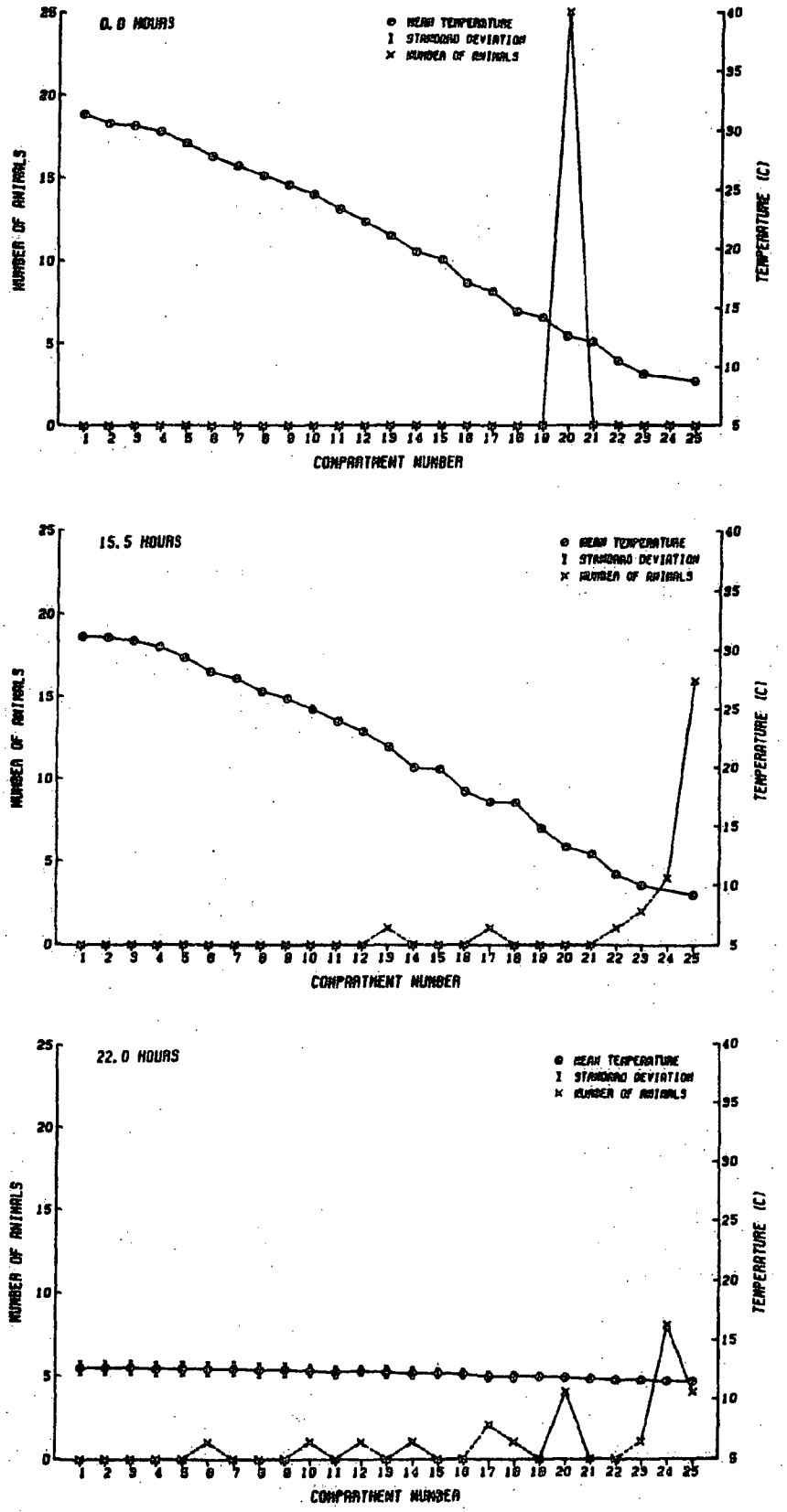


FIGURE 4.9.4-1

NUMBER OF ANIMALS AND TEMPERATURE VERSUS COMPARTMENT:
PAGURUS GRANOSIMANUS THERMAL PREFERENCE/AVOIDANCE
RUN 17M

tank; however, the majority of the animals were still at the end of the tank that had been cool.

In the second run on this species, the procedure was repeated with essentially similar results. By 17 hours, some animals were distributed up to 26 C (FIGURE 4.9.4-2) and by 73 hours, with an 11-14 C temperature range, some of the animals had redistributed themselves into the previously warm portion of the tank.

4.9.4.3 DISCUSSION

The Pagurus granosimanus tested in these experiments did not exhibit a preference response to temperature. An avoidance response was consistently evident, however, in that the animals did not move into locations where the temperature exceeded 20-26 C (68-79 F). The strong tendency of the animals to cluster at the cool end of the tank probably resulted from the natural tendency of these organisms to seek protected habitats in nature.

4.9.5 PAGURUS SAMUELIS THERMAL PREFERENCE/AVOIDANCE

According to Morris et al. (1980), the hermit crab Pagurus samuelis is abundant in tidepools on the protected rocky California coast mainly in the upper and middle intertidal regions but it extends into the subtidal where it occurs in Macrocystis holdfasts. Its geographical distribution extends from Vancouver Island, British Columbia, Canada to Punta Eugenia, Baja California, Mexico. It reaches a maximum carapace length of about 19 mm.

Although P. samuelis was not designated one of the representative important species (RIS) for the Diablo Canyon 316(a) demonstration, it is of common occurrence in the Diablo Canyon area. It is the most abundant tidepool hermit crab in southern and central California (Morris et al. 1980). More than 80 percent of the animals of reproductive size live in shells of Tegula. The diet of P. samuelis is varied and consists mostly of algal drift and dead animal tissue which is scavenged. Along with other hermit crabs, P. samuelis are an

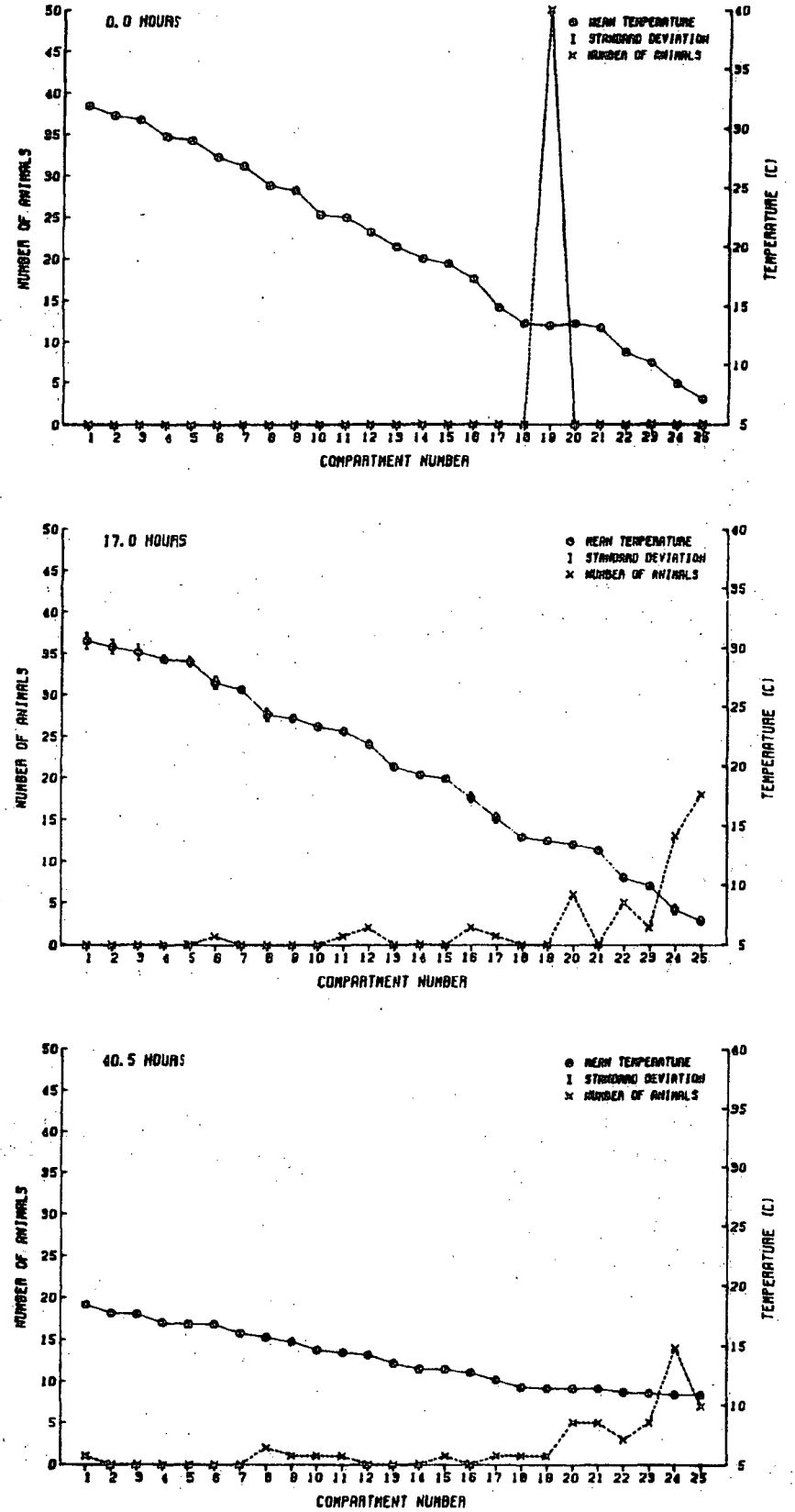


FIGURE 4.9.4-2

NUMBER OF ANIMALS AND TEMPERATURE VERSUS COMPARTMENT:
PAGURUS GRANOSIMANUS THERMAL PREFERENCE/AVOIDANCE
RUN 17W

important item in the diet of pile perch and are also eaten by sheephead, spotted kelpfish and striped kelpfish.

The objective of the thermal preference/avoidance experiments was to provide information on the behavioral response of P. samuelis to a thermal gradient, specifically, whether it exhibits preference for or avoidance to a particular temperature. The purpose was to provide information about the possible response of the species to the thermal plume, in particular, whether sublethal temperatures would have a major effect on its distribution in Diablo Cove.

4.9.5.1 METHODS

A summary of the methods employed in the thermal preference/avoidance experiments on P. samuelis was presented in SECTION 4.5.1 of this report. A more detailed account of the experiment procedure used in this experiment is presented in APPENDIX B (Procedure 317). The protocols for this experiment are included in APPENDIX C of this report (experiments 17u and 17v).

Adults, living in shells of 15-25 mm diameter, were used in these runs. They were collected during early 1979 from the vicinity of Diablo Cove.

While being held in the laboratory prior to experimentation, the animals were maintained in ambient temperature 4x8 ft flow-through tanks. During the holding period, the animals 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 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.

The 3-m gradient apparatus used for the experiment is described in APPENDIX A.

4.9.5.2 RESULTS

The first run on this species involved distributing the animals evenly across the gradient tank, two per compartment, with the entire tank at ambient seawater temperature (approximately 14 C). A gradient from 7-31 C was then established across the tank and by 23.5 hours into the experiment, the animals were still dispersed across the tank (FIGURE 4.9.5-1). The temperatures in the tank were then returned to 14-15 C and by 47.5 hours, the animals exhibited essentially the same distribution.

In the second run of this experiment, 50 specimens were introduced into the tank in the compartment that approximated ambient seawater temperature. A 7-31 C gradient was already established across the tank. By 19 hours, some dispersion of the animals had occurred, up to 29 C, but most of the animals remained near the compartment in which they were originally placed (FIGURE 4.9.5-2). The gradient was then removed and all compartments were at 14 C. By 52.5 hours, some of the animals had moved to the end of the tank that formerly was at 31 C. A tendency to cluster at the ends of the tank was evident at the 52.5 hour observation.

4.9.5.3 DISCUSSION

The specimens of Pagurus samuelis tested in this series of experiments did not exhibit a preference or an escape response to temperature. An avoidance response was evident, however, in that the animals did not move into locations where the temperature exceeded 29 C (84 F). A tendency of the animals to cluster at the ends of the tank probably reflects the natural behavior of the animals to seek protected habitats in nature. The experiment was conducted with a 12 hour light/12 hour dark photoperiod. According to Morris et al. (1980) P. samuelis is most active under dark conditions. The influence of the light/dark cycle on temperature avoidance, which could be significant, was not investigated in this experiment.

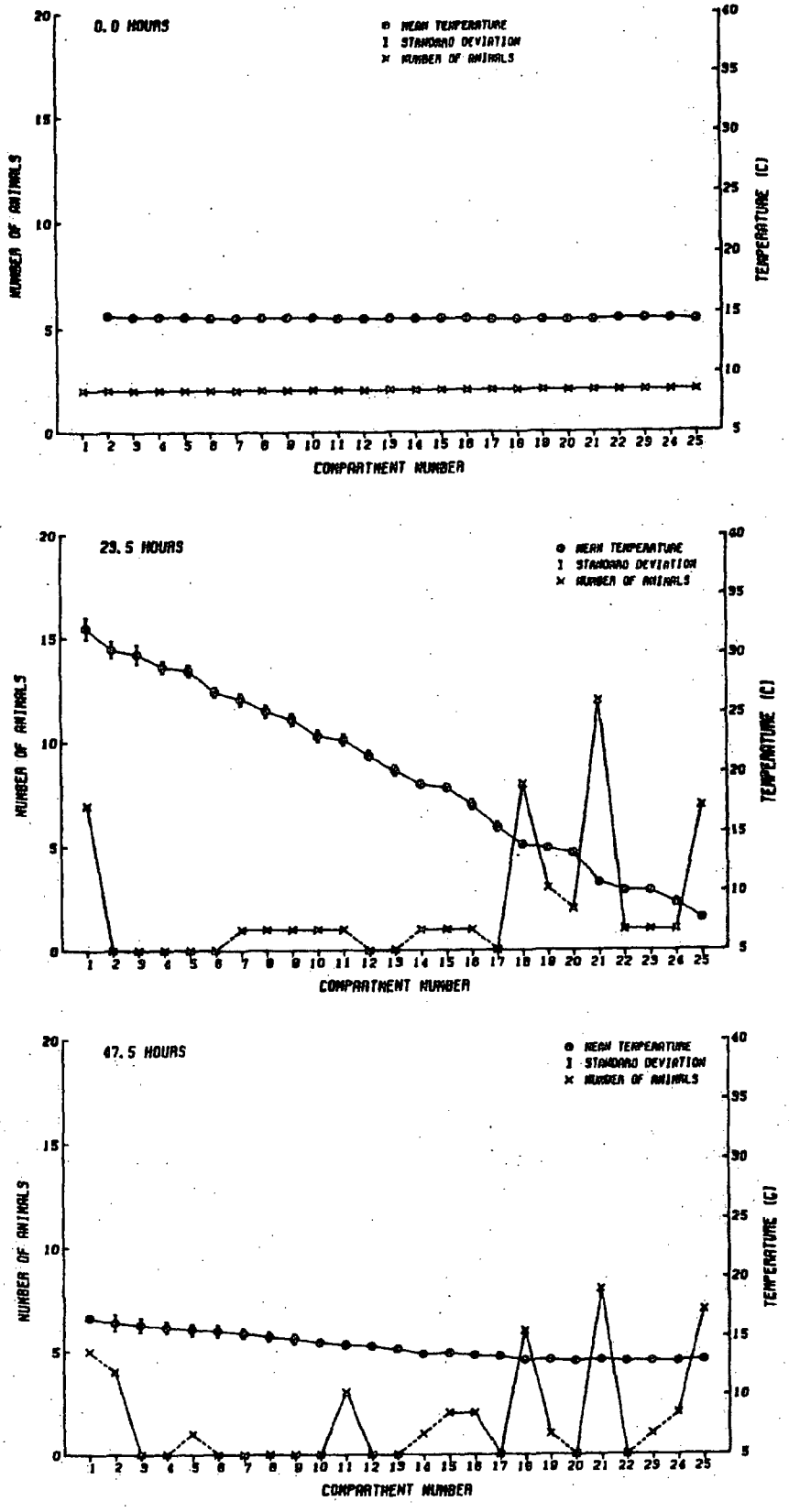


FIGURE 4.9.5-1

NUMBER OF ANIMALS AND TEMPERATURE VERSUS COMPARTMENT:
PAGURUS SAMUELIS THERMAL PREFERENCE/AVOIDANCE
RUN 17U

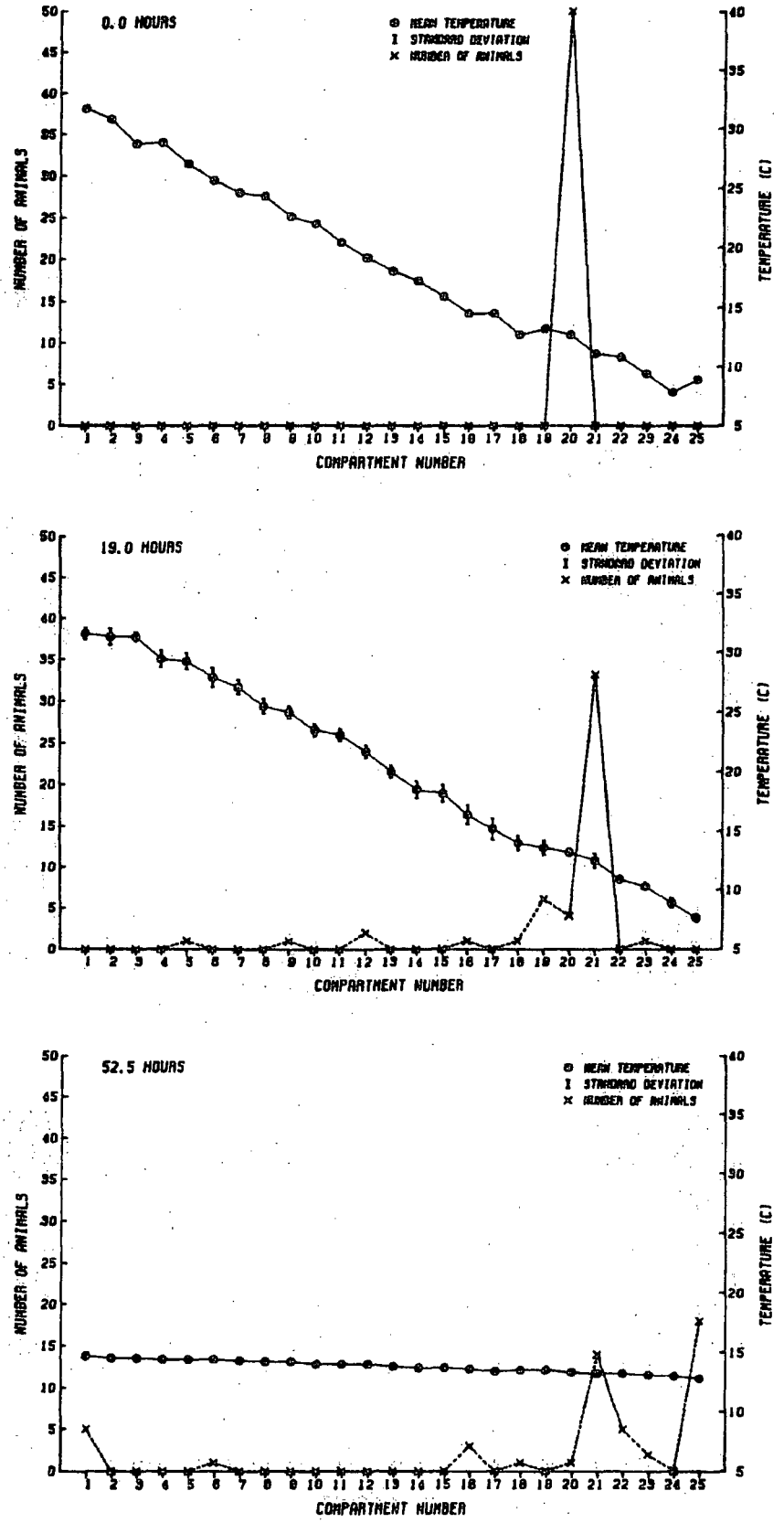


FIGURE 4.9.5-2

NUMBER OF ANIMALS AND TEMPERATURE VERSUS COMPARTMENT:
PAGURUS SAMUELIS THERMAL PREFERENCE/AVOIDANCE
RUN 17V

4.9.6 PATIRIA MINIATA THERMAL PREFERENCE/AVOIDANCE

According to Morris et al. (1980), Patiria miniata, the bat star, is common in California north of Point Conception. Its habitat is among rocks overgrown with surfgrass, larger algae, sponges and bryozoans in the low intertidal zone and subtidally to 290 m on rock, shell, gravel, hard sand and algae. Its geographical distribution extends from Sitka, Alaska to Baja California, Mexico. It reaches a maximum arm radius to 10 cm.

Although P. miniata was not designated one of the representative important species for the Diablo Canyon 316(a) demonstration, it is of common occurrence in the Diablo Canyon area and was included in the temperature preference/avoidance experiments because time and equipment were available.

The objective of the thermal preference/avoidance experiments was to provide information on the behavioral response of P. miniata to a thermal gradient, specifically, whether it exhibits preference for or avoidance to a particular temperature. The purpose was to provide information about the possible response of the species to the thermal plume, in particular, whether sublethal temperatures would have a major effect on its distribution in Diablo Cove.

4.9.6.1 METHODS

A summary of the methods employed in the thermal preference/avoidance experiments on P. miniata was presented in SECTION 4.5.1 of this report. A more detailed account of the experiment procedure used in this experiment is presented in APPENDIX B (Procedure 317). The protocols for this experiment are included in APPENDIX C of this report (experiments 17j and 17l).

Adult individuals, 13-15 cm in diameter, were used in these runs. They were collected during early 1979 from the vicinity of Diablo Cove. While being held in the laboratory prior to experimentation, the animals were maintained in ambient temperature 4x8 ft flow-through tanks. During the holding period, the animals

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 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.

The 3-m gradient apparatus used for the experiment is described APPENDIX A.

4.9.6.2 RESULTS

In the first run on P. miniata, the test animals were placed into several compartments at the center of the tank. The temperature was a uniform 13 C across the tank. There was no change in the temperature of the tank up to 5 hours into the experiment and by that time, a few individuals had moved to the end compartments. A thermal gradient was very gradually established and by 27 hours into the experiment, with a gradient of 10-27 C, there was essentially no change in the distribution of the test organisms in the tank (FIGURE 4.9.6-1).

The second run (171) involved placing 30 specimens in the tank in compartments approximating ambient temperature. A thermal gradient of 8-29 C was already established at that time. By 5 and 17.5 hours, the animals became dispersed over the tank up to 22-23 C. Following the 17.5 hour observation, the gradient was removed and all compartments were at ambient temperature. At 40.5 and again at 66 hours, the distribution of the animals had not changed (FIGURE 4.9.6-2).

4.9.6.3 DISCUSSION

The Patiria miniata tested in this series of experiments exhibited neither a preference nor an escape response to temperature. An avoidance response was evident, however, in that the animals did not move into locations where the temperature exceeded 22-23 C (72-73 F).

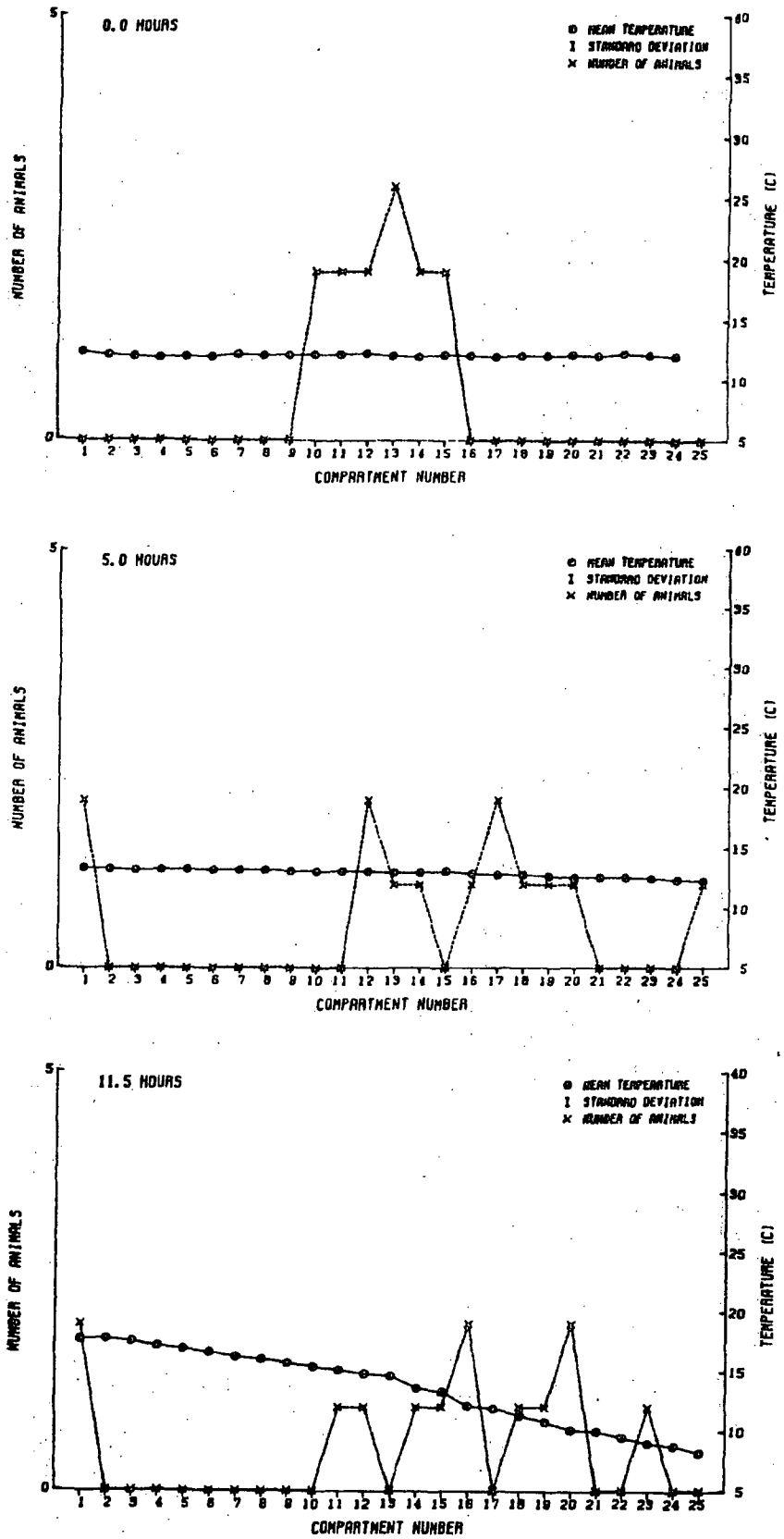


FIGURE 4.9.6-1

NUMBER OF ANIMALS AND TEMPERATURE VERSUS COMPARTMENT:
PATIRIA MINIATA THERMAL PREFERENCE/AVOIDANCE
RUN 17J

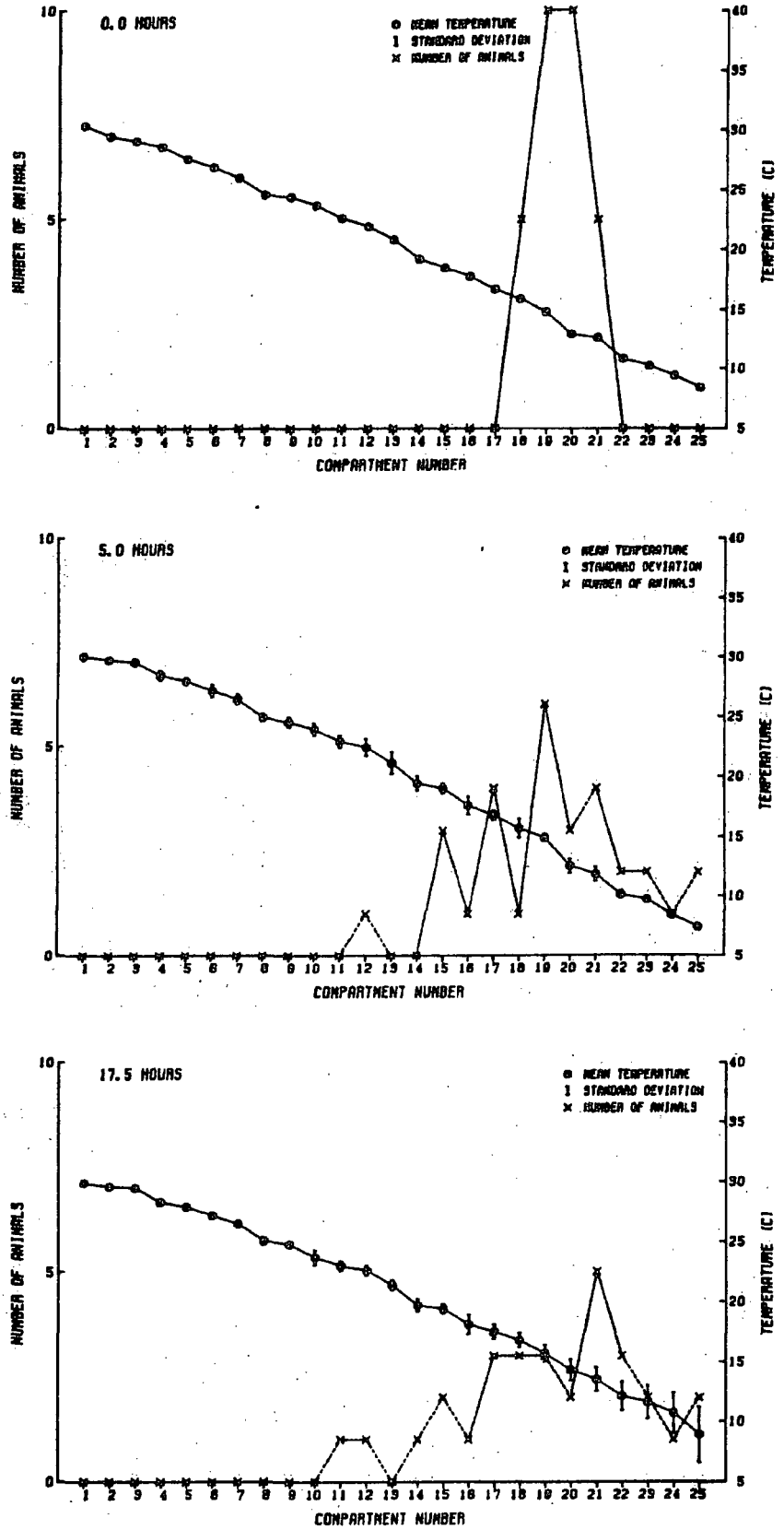


FIGURE 4.9.6-2

NUMBER OF ANIMALS AND TEMPERATURE VERSUS COMPARTMENT:
PATIRIA MINIATA THERMAL PREFERENCE/AVOIDANCE
RUN 17L

4.9.7 PISASTER GIGANTEUS THERMAL PREFERENCE/AVOIDANCE

According to Morris et al. (1980), The seastar Pisaster giganteus is common on rocks and pier pilings in the low intertidal region and into the subtidal region to 88 m depth. Its geographical distribution extends from Vancouver Island, British Columbia, Canada to Baja California, Mexico. It reaches arm radii to over 30 cm. Although P. giganteus was not designated one of the representative important species (RIS) for the Diablo Canyon 316(a) demonstration, it is of common occurrence in the Diablo Canyon area and was included in the temperature preference/avoidance experiments because time and equipment were available.

The objective of the thermal preference/avoidance experiments was to provide information on the behavioral response of P. giganteus to a thermal gradient, specifically, whether it exhibits preference for or avoidance to a particular temperature. The purpose was to provide information about the possible response of the species to the thermal plume, in particular, whether sublethal temperatures would have a major effect on its distribution in Diablo Cove.

4.9.7.1 METHODS

A summary of the methods employed in the thermal preference/avoidance experiments on P. giganteus was presented in SECTION 4.5.1 of this report. A more detailed account of the experiment procedure used in this experiment is presented in APPENDIX B (Procedure 317). The protocol for this experiment is included in APPENDIX C of this report (experiment 17n).

Adults, 16-20 cm in diameter, were used in this run. They were collected during early 1979 from the vicinity of Diablo Cove.

While being held in the laboratory prior to experimentation, the animals were maintained in ambient temperature 4x8 ft flow-through tanks. During the holding period, the animals 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 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.

The 3-m gradient apparatus used for the experiment is described in APPENDIX A. The procedure used to conduct the experiment involved establishing the experimental temperature gradient in the experimental tank and initiating the run by placing the test population in the compartment at ambient temperature. The organisms were then allowed to disperse in the tank for a period of hours without changing the temperature gradient. After at least one observation recording the position of the animals was made, the gradient was discontinued and the entire tank was maintained at ambient temperature. Again after a period of hours, a final observation was made to verify that the animals could disperse throughout the tank.

4.9.7.2 RESULTS

Twenty-two animals were placed in the gradient tank into compartments that approximated ambient seawater temperature. A thermal gradient ranging from 8-29 C was already established in the tank. By 18 hours (FIGURE 4.9.7-1) the animals had become distributed across the tank up to 27 C. Subsequently, the gradient was removed and all compartments were at 12-13 C. By 25 hours, the animals had redistributed themselves all across the tank.

4.9.7.3 DISCUSSION

The specimens of Pisaster giganteus tested in this experiment did not exhibit a preference response to temperature. An avoidance response was evident, however, in that the animals did not move into locations where the temperature exceeded 27 C (81 F).

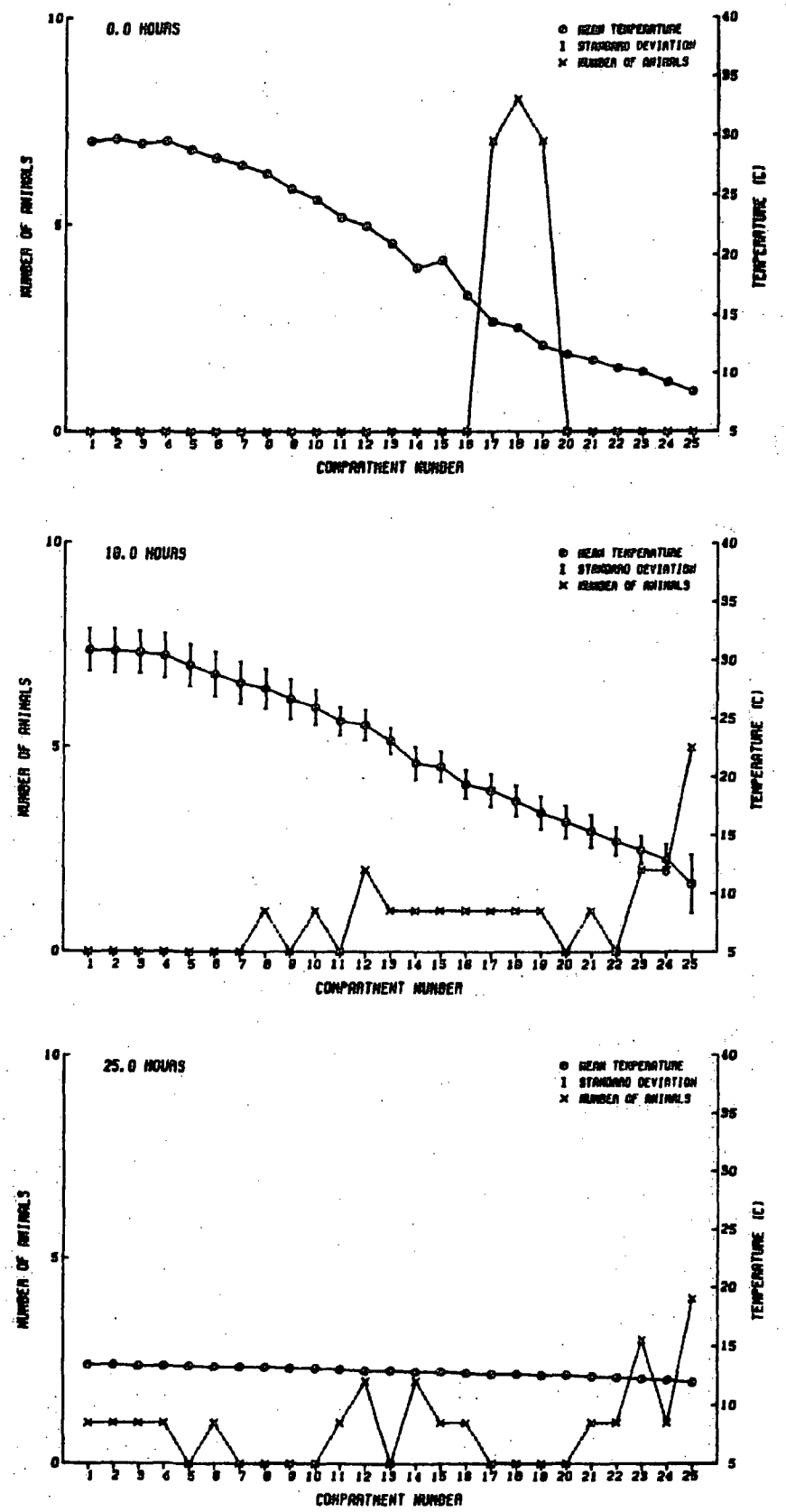


FIGURE 4.9.7-1

NUMBER OF ANIMALS AND TEMPERATURE VERSUS COMPARTMENT:
PISASTER GIGANTEUS THERMAL PREFERENCE/AVOIDANCE
RUN 17N

4.9.8 PISASTER OCHRACEUS THERMAL PREFERENCE/AVOIDANCE

According to Morris et al. (1980), Pisaster ochraceus, the ochre seastar, is common on the wave-swept rocky California coast in the low intertidal region and subtidally on rocks to 88 m. Its geographical distribution extends from Prince William Sound, Alaska to Point Sal, Santa Barbara County, California. It reaches an average arm radius of 14 cm.

Pisaster ochraceus was designated an RIS for the Diablo Canyon 316(a) demonstration. The literature on P. ochraceus was reviewed in a previous report (PGandE 1979b).

The objective of the thermal preference/avoidance experiments was to provide information on the behavioral response of P. ochraceus to a thermal gradient, specifically, whether it exhibits preference for or avoidance to a particular temperature. The purpose was to provide information about the possible response of the species to the thermal plume, in particular, whether sublethal temperatures would have a major effect on its distribution in Diablo Cove.

4.9.8.1 METHODS

A summary of the methods employed in the thermal preference/avoidance experiments on P. ochraceus was presented in SECTION 4.5.1 of this report. A more detailed account of the experiment procedure used in this experiment is presented in APPENDIX B (Procedure 317). The protocol for this experiment (17n) is included in APPENDIX C of this report.

Adult specimens of P. ochraceus ranging from 16 to 20 cm in diameter were used in this run. They were collected during early 1979 from the vicinity of Diablo Cove.

While being held in the laboratory prior to experimentation, the animals were maintained in ambient temperature 4x8 ft flow-through tanks. During the

holding period, the animals 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 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.

The 3-m gradient apparatus used for the experiment is described in APPENDIX A. The procedure used to conduct the experiment involved establishing the experimental temperature gradient in the experimental tank and initiating the run by placing the test population in the compartment at the acclimation temperature for the test organisms. The organisms were then allowed to disperse in the tank for a period of hours without changing the temperature gradient. After at least one observation recording the position of the animals was made, the gradient was discontinued and the entire tank was maintained at the acclimation temperature of the organisms. Again after a period of hours, a final observation was made to verify that the animals could disperse throughout the tank.

4.9.8.2 RESULTS

Twenty-two animals were placed in the gradient tank in compartments approximating ambient seawater temperature. A gradient of 8-29 C was already established across the gradient tank. By 18 hours (FIGURE 4.9.8-1) the test animals had distributed themselves into two groups, one toward the cool end of the tank and the other toward the warm end up to 25 C. Subsequently, the gradient was removed and some of the animals moved into the warm portion of the tank from which they had been excluded.

4.9.8.3 DISCUSSION

The Pisaster ochraceus tested in this experiment did not exhibit a preference response to temperature. An avoidance response was consistently evident,

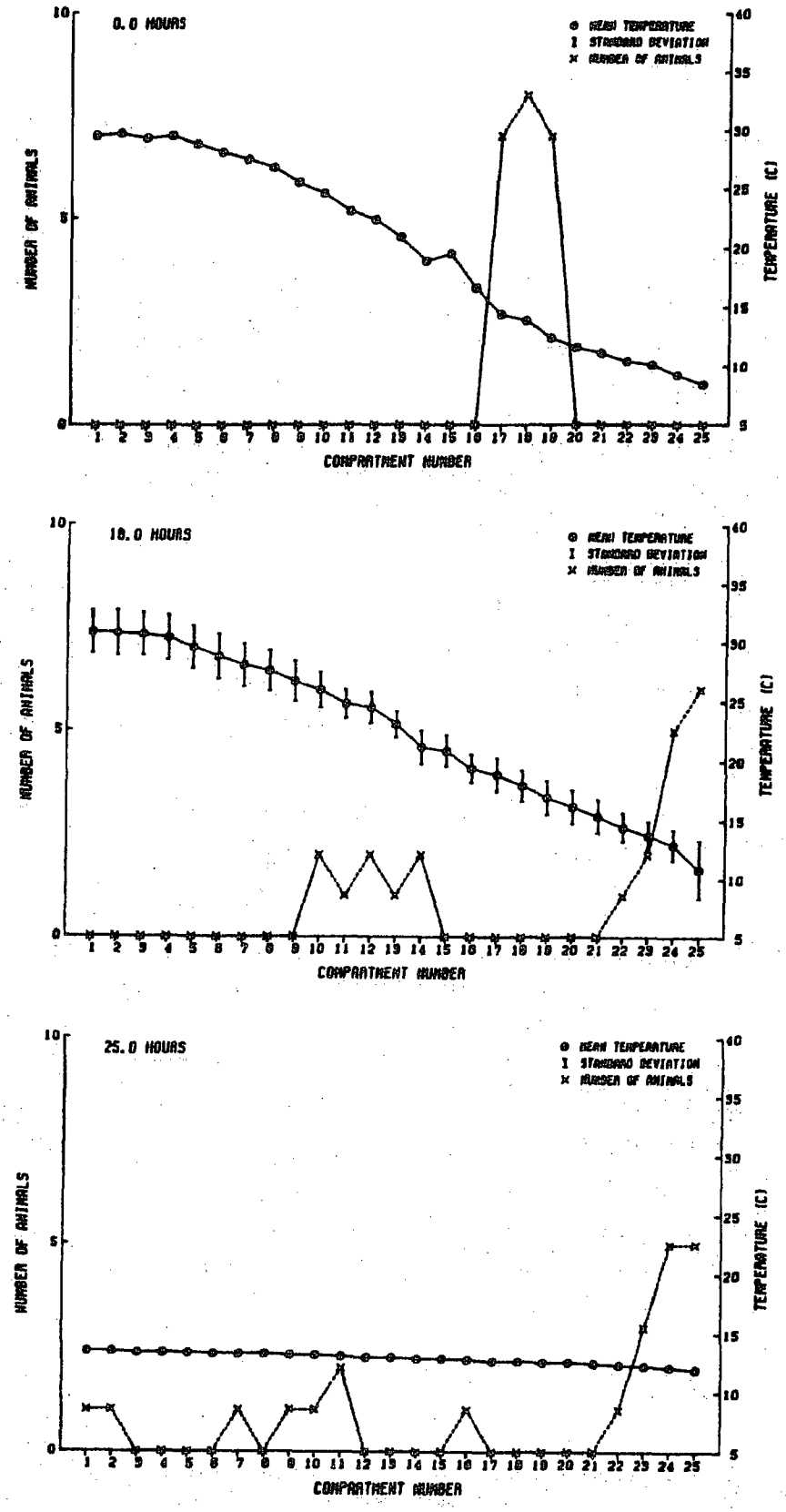


FIGURE 4.9.8-1

NUMBER OF ANIMALS AND TEMPERATURE VERSUS COMPARTMENT:
PISASTER OCHRACEUS THERMAL PREFERENCE/AVOIDANCE

however, in that the animals did not move into locations where the temperature exceeded 25 C (77 F).

4.9.9 PUGETTIA PRODUCTA THERMAL PREFERENCE/AVOIDANCE

According to Morris et al. (1980), The kelp crab Pugettia producta is common among rocks and brown algae in the low intertidal region on the rocky California coast. Its geographical distribution extends from Prince of Wales Island, Alaska to Punta Asuncion, Baja California, Mexico.

Although P. producta was not designated one of the representative important species for the Diablo Canyon 316(a) demonstration, it is of common occurrence in the Diablo Canyon area and was included in the temperature preference/avoidance experiments because time and equipment were available.

The objective of the thermal preference/avoidance experiments was to provide information on the behavioral response of P. producta to a thermal gradient, specifically, whether it exhibits preference for or avoidance to a particular temperature. The purpose was to provide information about the possible response of the species to the thermal plume, in particular, whether sublethal temperatures would have a major effect on its distribution in Diablo Cove.

4.9.9.1 METHODS

A summary of the methods employed in the thermal preference/avoidance experiments on P. producta was presented in SECTION 4.5.1 of this report. A more detailed account of the experiment procedure used in this experiment is presented in APPENDIX B (Procedure 317). The protocols for this experiment are included in APPENDIX C of this report (experiments 17p and 17q).

Both juvenile and adult specimens of P. producta (carapace widths 10-40 mm) were used in these runs. They were collected during early 1979 from the vicinity of Diablo Cove.

While being held in the laboratory prior to experimentation, the animals were maintained in ambient temperature 4x8 ft flow-through tanks and were fed to excess with Macrocystis. During the holding period, the animals 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 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.

The 3-m gradient apparatus used for the experiment is described in APPENDIX A. Two procedures were used in the preference/avoidance experiments on P. producta. In the first run, animals were allowed to disperse over the tank at ambient temperature and a gradient was subsequently established to evaluate their escape response. In the second run, the procedure involved establishing the experimental temperature gradient in the experimental tank and initiating the run by placing the test population in the compartment at the acclimation temperature for the test organisms. The organisms were then allowed to disperse in the tank for a period of hours without changing the temperature gradient. After at least one observation recording the position of the animals was made, the gradient was discontinued and the entire tank was maintained at the acclimation temperature of the organisms. Again after a period of hours, a final observation was made to verify that the animals could disperse throughout the tank.

4.9.9.2 RESULTS

In the first run (17p) 25 specimens were placed in the center of the tank which was uniformly at ambient seawater temperature. After 15 hours, the animals had distributed themselves over the tank with a tendency to cluster at the end compartments. Subsequently, a thermal gradient was established ranging from 7-29 C and by 38 hours, the animals did not occur in compartments with

temperatures exceeding 25 C (FIGURE 4.9.9-1). A tendency to cluster at the cool end compartment was still evident.

In the second run (17q) 25 specimens were placed into the tank in a compartment that approximated ambient seawater temperature. A 7-30 C gradient was already established at that time. After 14 hours, the animals had become distributed across the tank up to 21 C (FIGURE 4.9.9-2). Subsequently, the gradient was removed and the temperature was 13-14 C across the tank. By 47 hours, a few of the animals moved into the unoccupied portion of the tank, but for the most part they remained in essentially the same distribution.

4.9.9.3 DISCUSSION

The kelp crab Pugettia producta tested in these two runs did not exhibit a preference response to temperature. An escape response was detected in the first run on this species when the animals retreated from areas exceeding 25 C (77 F) which earlier had been occupied under cooler temperatures. An avoidance response was also evident in that the animals did not move into locations where the temperature exceeded 21 C (70 F). A tendency of the animals to cluster at the ends of the tank in both runs probably resulted from a natural tendency of the animals to seek protected habitats on macroscopic algae.

4.9.10 PYCNOPODIA HELIANTHOIDES THERMAL PREFERENCE/AVOIDANCE

According to Morris et al. (1980), Pycnopodia helianthoides, the sunflower star, occurs frequently in regions rich in seaweeds from the low intertidal zone down to 435 m depth on the rocky California coast. Its geographical distribution extends from Unalaska Island, Alaska to San Diego, California. It reaches a maximum arm radius of 40 cm or more. It is the largest, heaviest and most active of the Pacific Coast seastars.

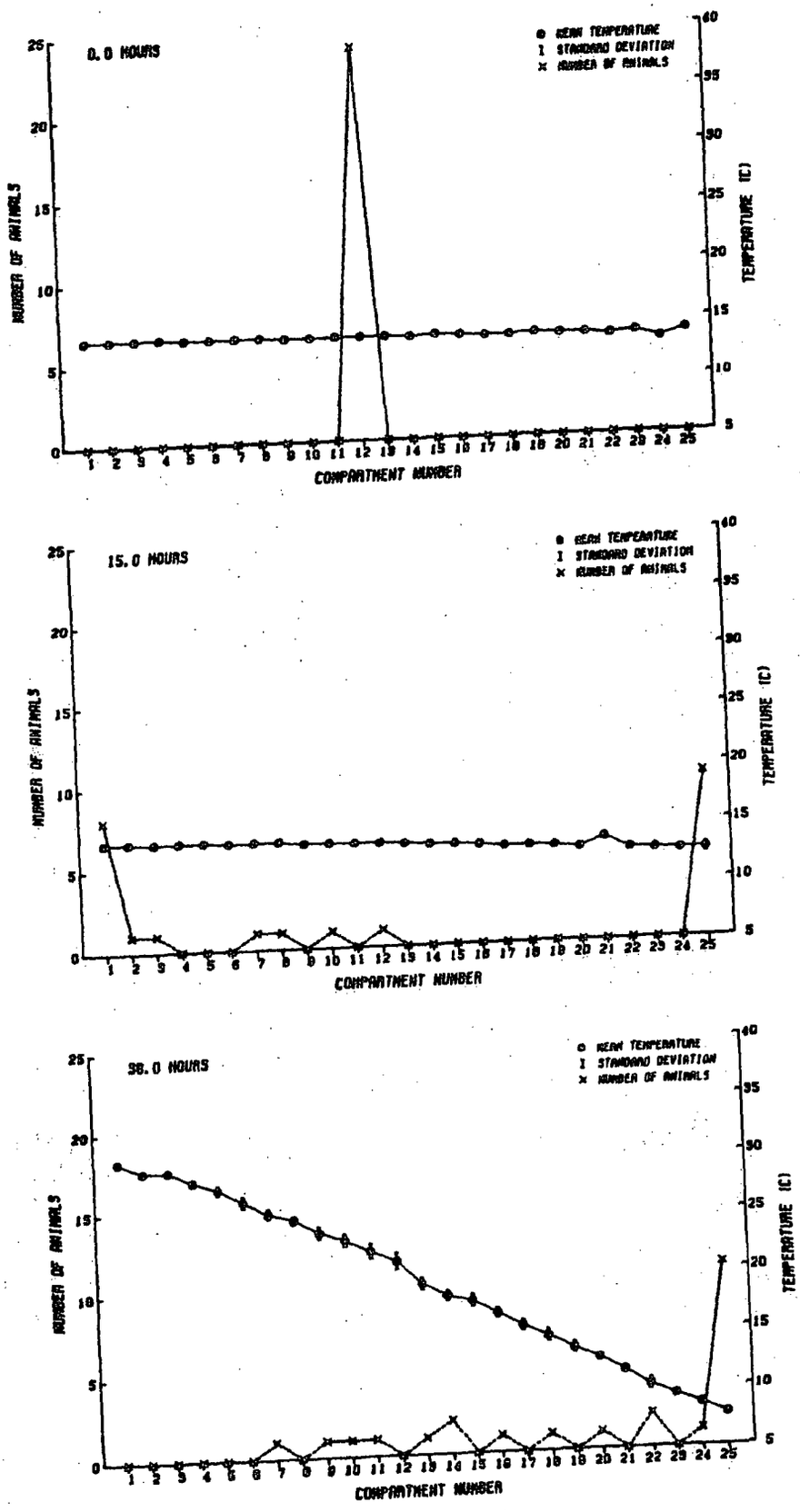


FIGURE 4.9.9-1

NUMBER OF ANIMALS AND TEMPERATURE VERSUS COMPARTMENT:
PUGETTIA PRODUCTA THERMAL PREFERENCE/AVOIDANCE
RUN 17P

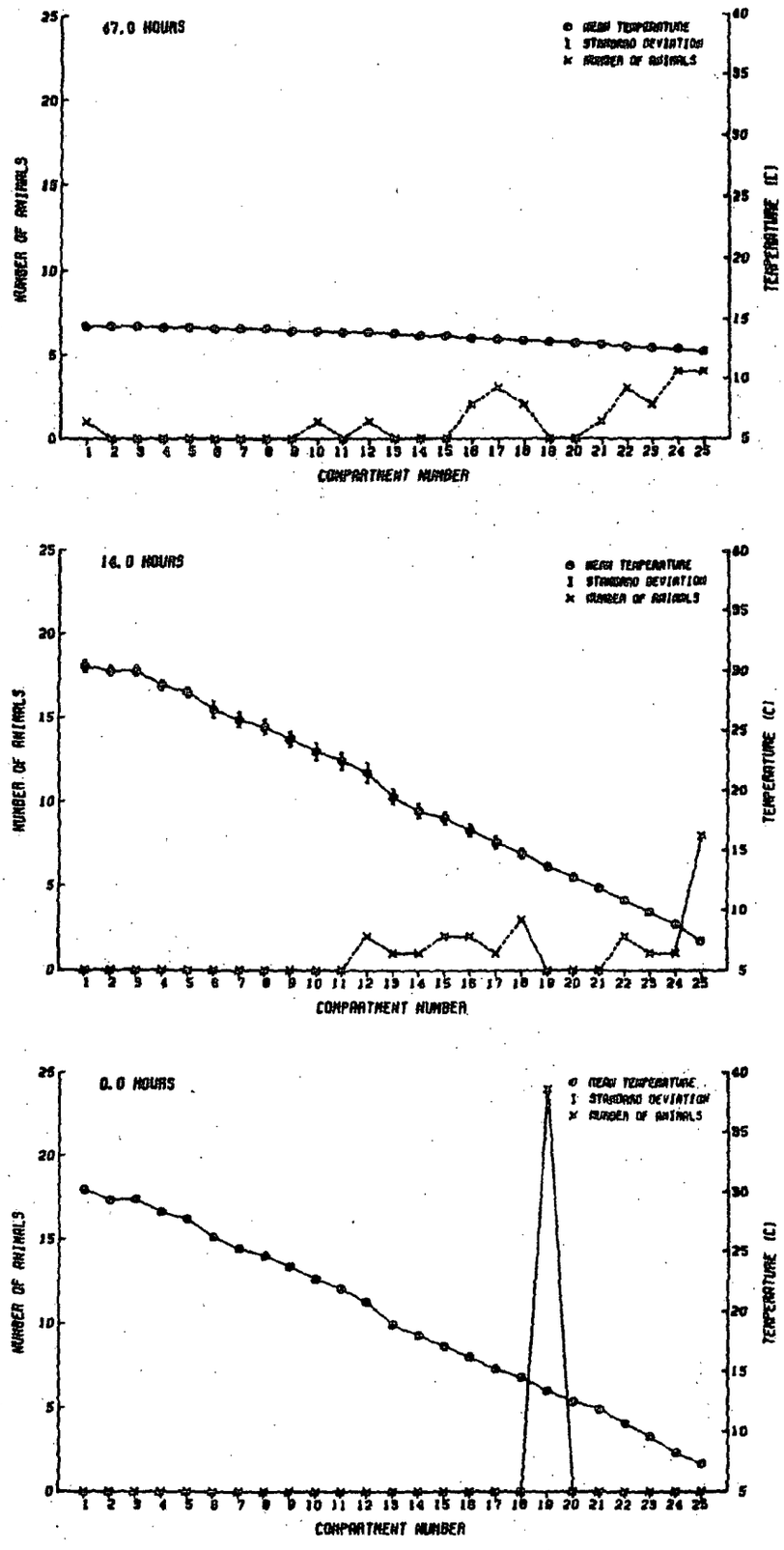


FIGURE 4.9.9-2

NUMBER OF ANIMALS AND TEMPERATURE VERSUS COMPARTMENT:
PUGETTIA PRODUCTA THERMAL PREFERENCE/AVOIDANCE
RUN 17Q

Pycnopodia helianthoides was designated a representative important species (RIS) for the Diablo Canyon 316(a) demonstration. The literature on P. helianthoides was reviewed in a previous report (PGandE 1979b).

The objective of the thermal preference/avoidance experiments was to provide information on the behavioral response of P. helianthoides to a thermal gradient, specifically, whether it exhibits preference for or avoidance to a particular temperature. The purpose was to provide information about the possible response of the species to the thermal plume, in particular, whether sublethal temperatures would have a major effect on its distribution in Diablo Cove.

4.9.10.1 METHODS

A summary of the methods employed in the thermal preference/avoidance experiments on P. helianthoides was presented in SECTION 4.5.1 of this report. A more detailed account of the experiment procedure used in this experiment is presented in APPENDIX B (Procedure 317). The protocols for this experiment are included in APPENDIX C of this report (experiments 17x and 17y).

Individuals tested in these runs ranged from 6 to 25 cm in diameter. They were collected during early 1979 from the vicinity of Diablo Cove.

While being held in the laboratory prior to experimentation, the animals were maintained in ambient temperature 4x8 ft flow-through tanks. During the holding period, the animals 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 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.

The 3-m gradient apparatus used for the experiment is described in APPENDIX A. Two procedures were used in the preference/avoidance

experiments on P. helianthoides. In the first run, animals were allowed to disperse over the tank at ambient temperature to verify that they would disperse across the tank when temperature is not influencing their behavior. In the second run, the procedure involved establishing the experimental temperature gradient in the experimental tank and initiating the run by placing the test population in the compartment at the acclimation temperature for the test organisms. The organisms were then allowed to disperse in the tank for a period of hours without changing the temperature gradient. After at least one observation recording the position of the animals was made, the gradient was discontinued and the entire tank was maintained at ambient temperature. Again after a period of hours, a final observation was made to verify that the animals could disperse throughout the tank.

4.9.10.2 RESULTS

In the first run, 20 animals were placed into the gradient tank with a uniform temperature of 13-14 C. By 24.5 hours, they had distributed themselves uniformly across the tank. In the second run, another 20 specimens were introduced into the tank locations approximating ambient seawater temperature, with a thermal gradient of 7-26 C already established across the tank. By 6 hours (FIGURE 4.9.10-1) the animals had become distributed across the tank up to 21 C. At that time, it was noted that some water had leaked from the tank. This was corrected, the water level was brought back up and the animals were replaced into the tank at ambient temperature by 7 hours into the experiment. After 22.5 hours, they once again distributed themselves up to 21 C. The gradient was subsequently removed so that all compartments were at 13-14 C and at 49 hours into the experiment, the animals were uniformly distributed across the tank.

4.9.10.3 DISCUSSION

The Pycnopodia helianthoides specimens tested in these runs did not exhibit a preference response to temperature. An avoidance response was consistently

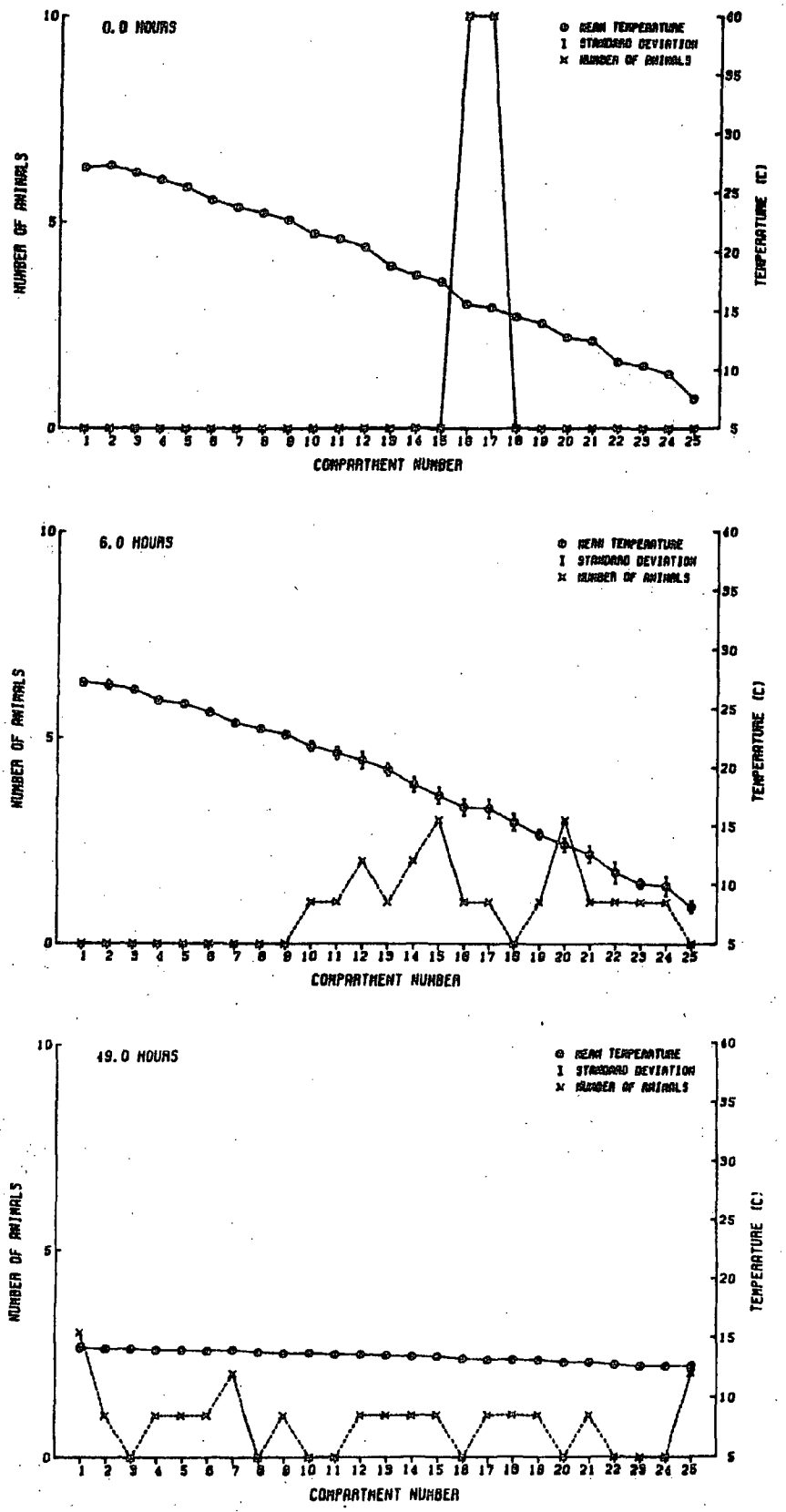


FIGURE 4.9.10-1

NUMBER OF ANIMALS AND TEMPERATURE VERSUS COMPARTMENT:
PYCNOPODIA HELIANTHOIDES THERMAL PREFERENCE/AVOIDANCE
RUN 17Y

evident, however, in that the animals did not move into locations where the temperature exceeded 21 C (70 F).

4.9.11 TEGULA BRUNNEA THERMAL PREFERENCE/AVOIDANCE

According to Morris et al. (1980), Tegula brunnea, the brown turban snail, is abundant on the rocky California coast in the low intertidal region and on upper blades and stipes of kelp offshore. Its geographical distribution extends from Cape Arago, Oregon to Santa Barbara Island, Channel Islands, California. It reaches a maximum shell diameter of 30 mm.

Tegula brunnea was designated an RIS for the Diablo Canyon 316(a) demonstration. The literature on T. brunnea was reviewed in a previous report (PGandE 1979b).

The objective of the thermal preference/avoidance experiments was to provide information on the behavioral response of T. brunnea to a thermal gradient, specifically, whether it exhibits preference for or avoidance to a particular temperature. The purpose was to provide information about the possible response of the species to the thermal plume, in particular, whether sublethal temperatures would have a major effect on its distribution in Diablo Cove.

4.9.11.1 METHODS

A summary of the methods employed in the thermal preference/avoidance experiments on T. brunnea was presented in SECTION 4.5.1 of this report. A more detailed account of the experiment procedure used in this experiment is presented in APPENDIX B (Procedure 317). The protocols for this experiment are included in APPENDIX C of this report (experiments 17a to c and e to i).

Both juvenile (less than 15 mm shell diameter) and adult (greater than 15 mm diameter) specimens of T. brunnea were used in these runs. They were collected during early 1979 from the vicinity of Diablo Cove.

A total of eight runs were made on this species. A summary of the run data is presented in TABLE 4.9.11-1. While being held in the laboratory prior to experimentation, the animals were fed to excess with Macrocystis and maintained in ambient temperature 4x8 ft flow-through tanks. During the holding period, the animals 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 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.

The 3-m gradient apparatus used for the experiment is described in APPENDIX A. Because these experiments represented the first attempts to study the behavioral responses of marine invertebrates to a thermal gradient, there were no standard experimental protocols to follow. From the initial runs, it was determined that the most efficient method of conducting the experiment was to establish the experimental temperature gradient in the experimental tank and to initiate the run by placing the test population in the compartment at the acclimation temperature for the test organisms. The organisms were then allowed to disperse in the tank for a period of hours without changing the temperature gradient. After at least one observation recording the position of the animals was made, the gradient was discontinued and the entire tank was maintained at the acclimation temperature of the organisms. Again after a period of hours, a final observation was made to verify that the animals could disperse throughout the tank. To a certain extent, the results presented reflect trials of various protocols used in determining the best approach. Once the experimental protocol was established, groups of animals acclimated to different temperatures were subjected to testing to determine the effects of acclimation on temperature avoidance.

It was possible to include more than one species simultaneously in the invertebrate thermal avoidance experiments, and this was done to the extent possible to increase efficiency. To be grouped together, species had to be compatible with

TABLE 4.9.11-1

PROTOCOL SUMMARY FOR TEGULA BRUNNEA
THERMAL PREFERANCE/AVOIDANCE

Run	Sample Size	Acclimation Temperature	Gradient Status (Temperature, C)		
			Initial	First Change	Second Change
17a[1]	25	ambient	ambient	7-18	6-22
17b[1]	25	ambient	6-21	--	--
17c[2]	30	ambient	8-29	8-22	--
17e[2]	30	ambient	ambient	8-26	13-17
17f[3]	30	ambient	8-30	13-14	--
17g[3]	30	18	7-31	--	--
17h[3]	30	18	8-37	18	--
17i[3]	31	21	7-37	21	--

1 Run made with Haliotis cracherodii also in the tank
2 Run made with Haliotis cracherodii and Haliotis rufescens also in the tank
3 Run made with Haliotis cracherodii, T. funebralis and Haliotis rufescens also in the tank

each other (not mutual prey or predators) and of a size and mobility that precluded physical obstruction of movement in the tank.

4.9.11.2 RESULTS

A summary of the protocols for the runs on T. brunnea is presented in TABLE 4.9.11-1. The data are presented in the graphs shown in FIGURES 4.9.11-1 to 4.9.11-6.

Runs 17a and 17b

The first run on this species was preliminary and involved placing the animals into the gradient tank at their approximate acclimation temperature. By 16 hours into the experiment, the animals had dispersed over the tank and subsequent establishment of a thermal gradient (7-18 C by 24 hours and 6-22 C by 40 hours) did not substantially alter this distribution (FIGURE 4.9.11-1) In the second run on this species, the animals were placed into the tank with a thermal gradient already established, ranging from 6-22 C. The animals became dispersed over the tank and remained similarly distributed after 65 hours.

Run 17c

The ambient acclimated test animals were introduced into the tank at their acclimation temperature with a thermal gradient of 7-30 C established (FIGURE 4.9.11-2). At 16 hours into the run, the animals had dispersed over that portion of the tank in which the temperature was at or below 20 C. After the 16 hour observation, the gradient range was reduced to 8-22 C and at 40 hours, the animals had dispersed across the entire tank.

Run 17e

The animals were introduced into compartment 13 with the tank at about 13 C (no thermal gradient) and by 17.5 hours had dispersed over the entire tank. A gradient was then induced ranging from 8-26 C and at the 26 hour observation

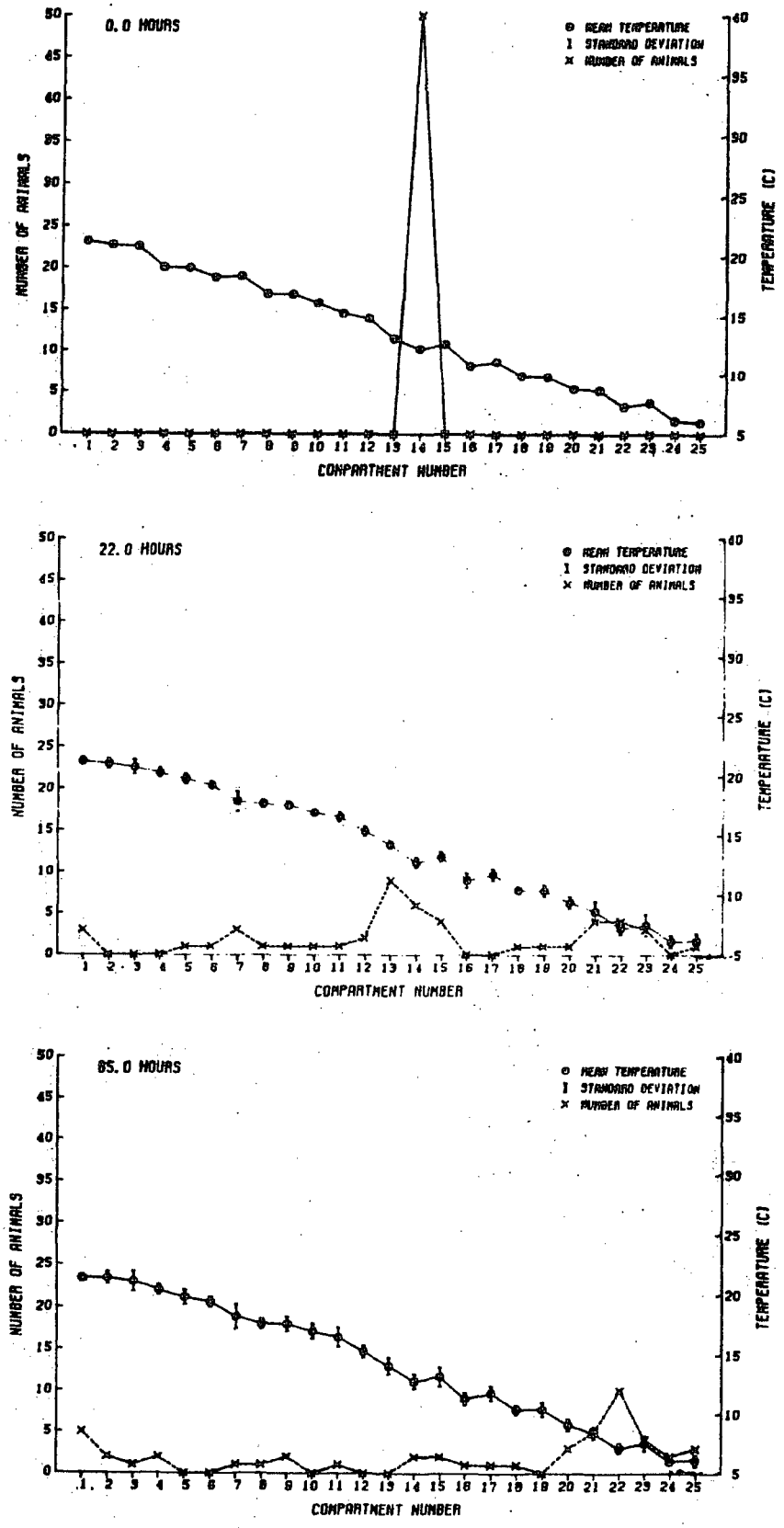


FIGURE 4.9.11-1

NUMBER OF ANIMALS AND TEMPERATURE VERSUS COMPARTMENT:
TEGULA BRUNNEA THERMAL PREFERENCE/AVOIDANCE
RUN 17B

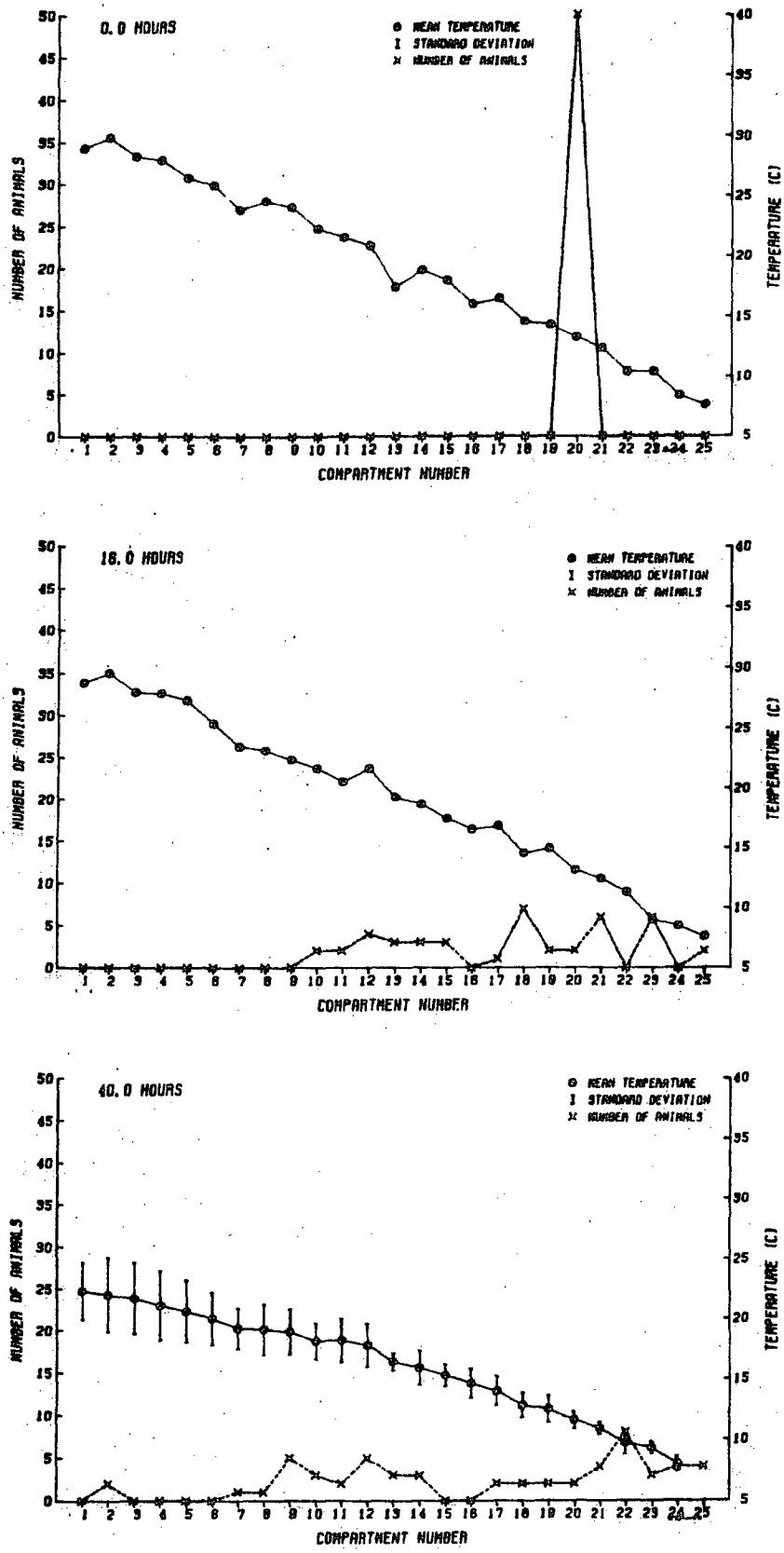


FIGURE 4.9.11-2

NUMBER OF ANIMALS AND TEMPERATURE VERSUS COMPARTMENT:
TEGULA BRUNNEA THERMAL PREFERENCE/AVOIDANCE
RUN 17C

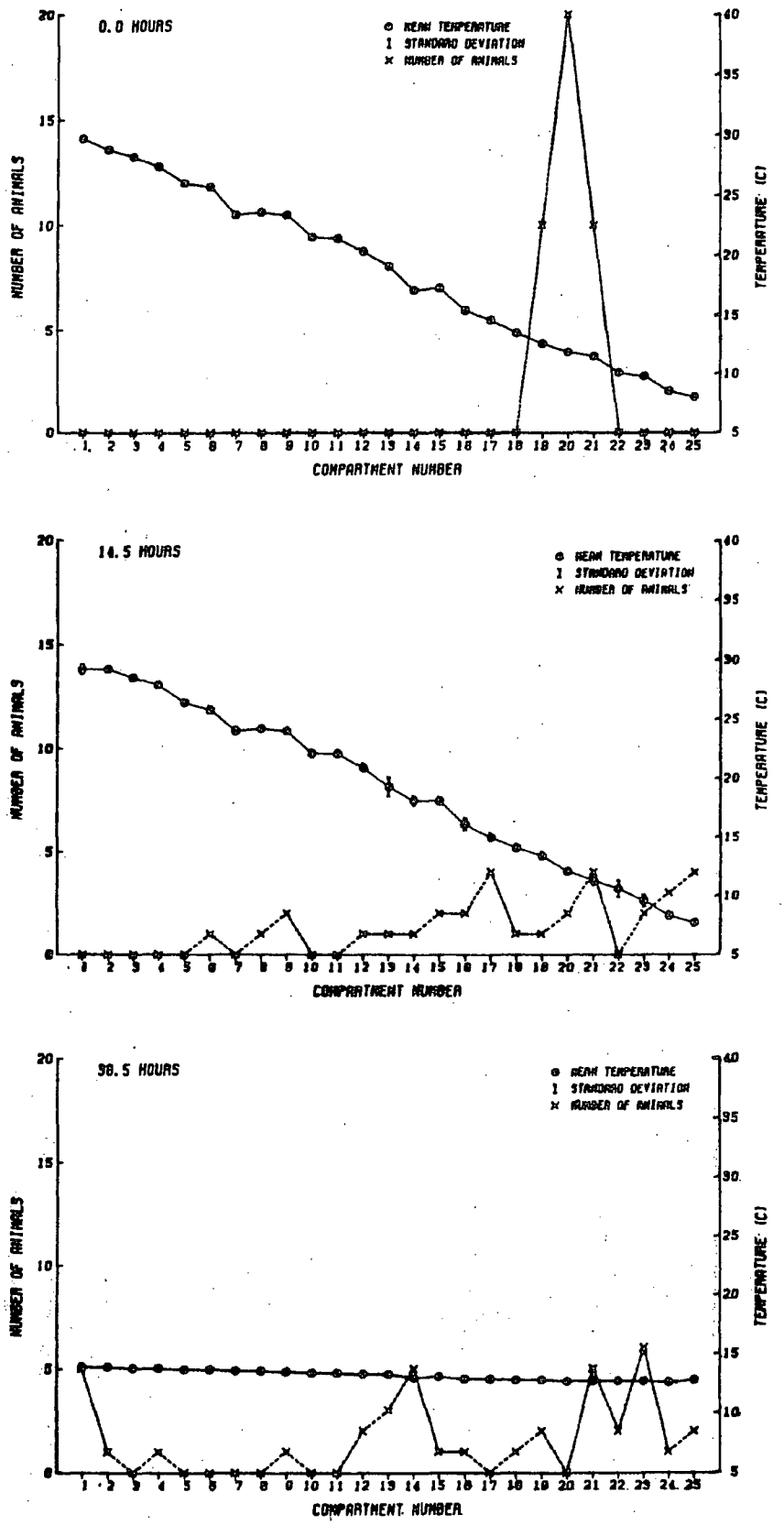
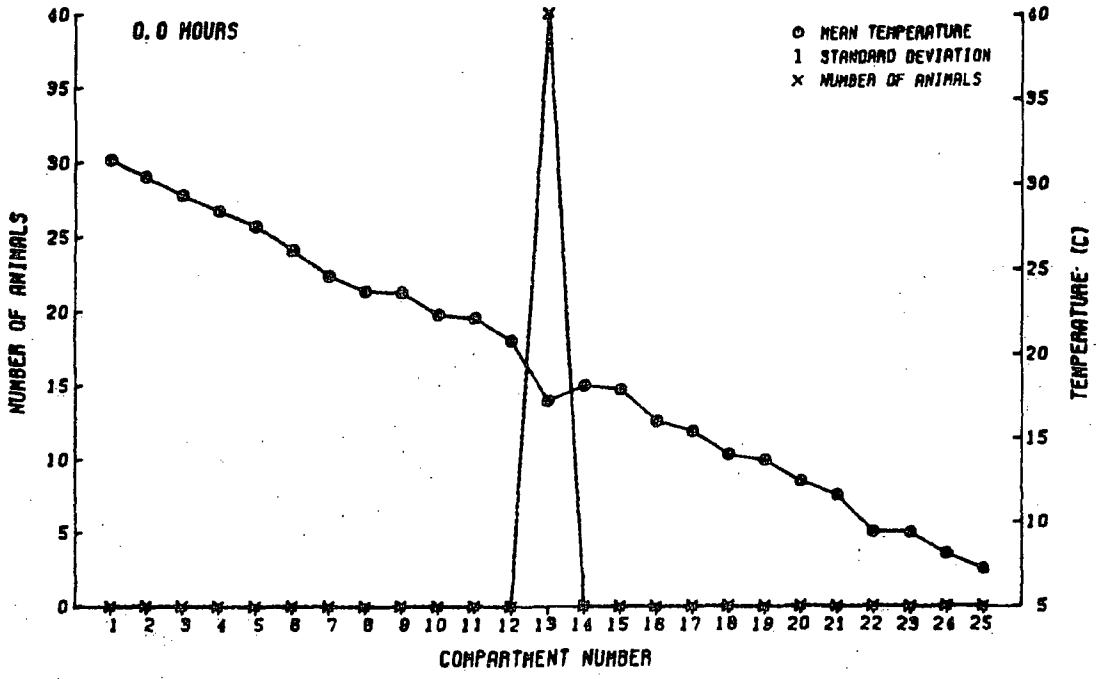


FIGURE 4.9.11-3

NUMBER OF ANIMALS AND TEMPERATURE VERSUS COMPARTMENT:
TEGULA BRUNNEA THERMAL PREFERENCE/AVOIDANCE
RUN 17F



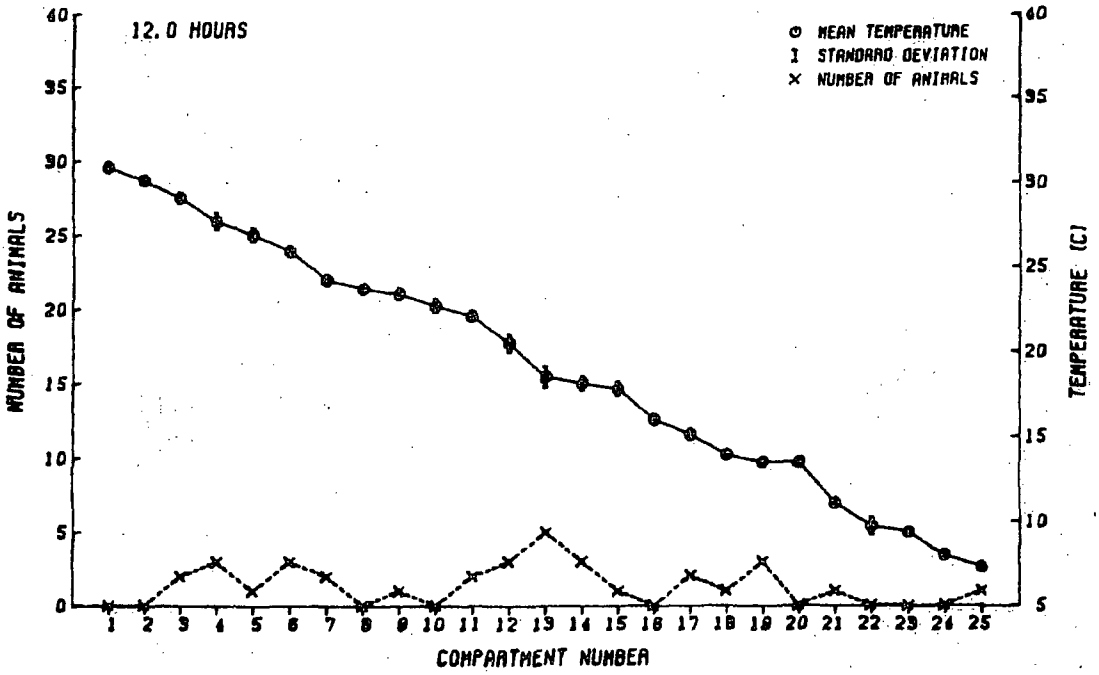


FIGURE 4.9.11-4

NUMBER OF ANIMALS AND TEMPERATURE VERSUS COMPARTMENT:
TEGULA BRUNNEA THERMAL PREFERENCE/AVOIDANCE
RUN 17G

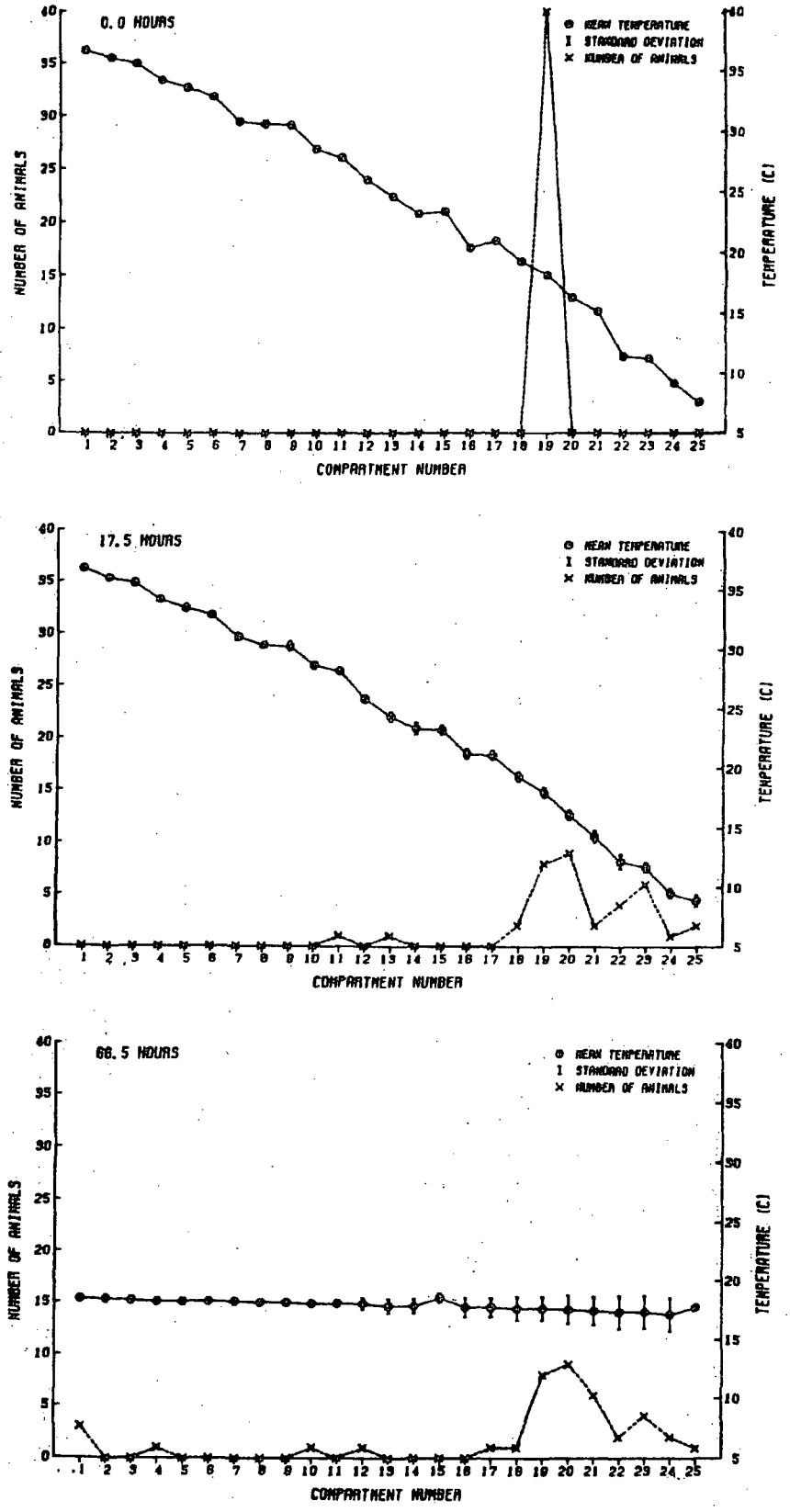


FIGURE 4.9.11-5

NUMBER OF ANIMALS AND TEMPERATURE VERSUS COMPARTMENT:
TEGULA BRUNNEA THERMAL PREFERENCE/AVOIDANCE
RUN 17H

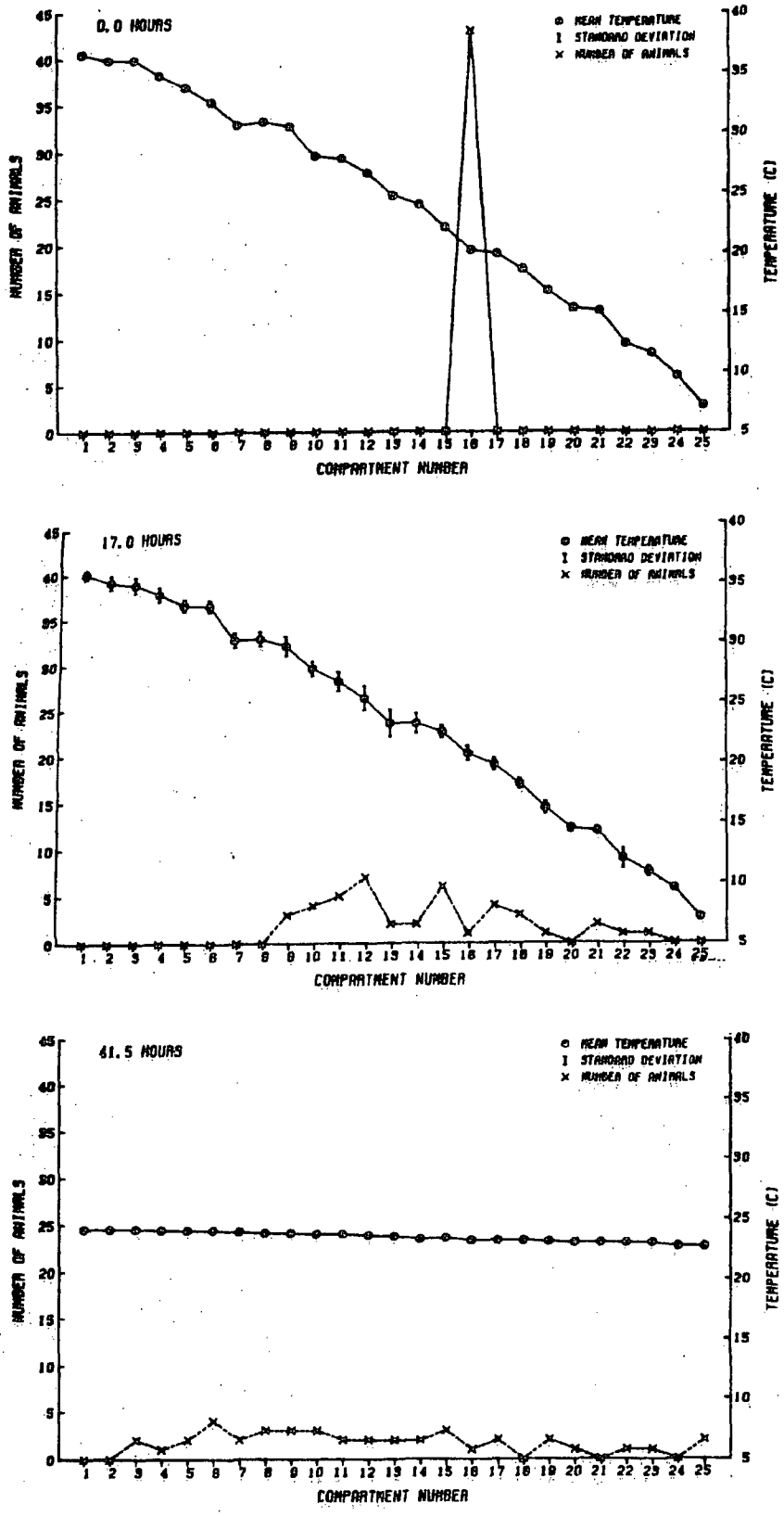


FIGURE 4.9.11-6

NUMBER OF ANIMALS AND TEMPERATURE VERSUS COMPARTMENT:
TEGULA BRUNNEA THERMAL PREFERENCE/AVOIDANCE
RUN 171

this gradient had not significantly affected their distribution in the tank. The gradient was then reduced to 13-18 C and at the 40 hour observation the distribution of the animals remained essentially the same.

Run 17f

The animals were introduced into the tank with a gradient already established (8-29 C). By 14.5 hours, the animals had dispersed over the tank up to 25 C (FIGURE 4.9.11-3). When the gradient was subsequently reduced to 13-14 C, by 38.5 hours the animals redistributed themselves over the portion of the tank that was previously excluded.

Run 17g

Prior to this run, all animals had been ambient acclimated. In this run, 18 C-acclimated animals from the snail growth experiment were placed at their acclimation temperature within a range of 7-31 C. At 12 hours, they had dispersed over the tank up to 28 C (FIGURE 4.9.11-4).

Run 17h

This run involved a second test of the 18 C-acclimated animals. A 7-37 C gradient was established prior to placement of the animals in the tank. By 17.5 hours the animals had dispersed over the tank up to 28 C (FIGURE 4.9.11-5). The gradient was subsequently reduced to 17-18 C and by 66.5 hours some animals had moved into compartments not occupied at 17.5 hours.

Run 17i

In this final run on T. brunnea, 21 C-acclimated animals from the snail growth experiment were introduced into the tank with a 7-36 C gradient. At the 17 hour observation, they had dispersed across the tank up to 29 C (FIGURE 4.9.11-6). It was noted, however, that two of the three animals that were situated in the

compartment above 28 C had expired. The gradient was subsequently reduced to 23-24 C and by 41.5 hours the animals had dispersed across the entire tank.

4.9.11.3 DISCUSSION

In general, the Tegula brunnea tested in this series of experiments did not exhibit a preference response to temperature. An avoidance response was consistently evident, however, in that the animals did not move into locations where the temperature exceeded a certain value. This "avoidance temperature" was positively related to the acclimation temperature of the animals prior to testing (20-25 C or 68-77 F for ambient acclimated and 28 C or 82 F for 18-21 C- or 64-70 F-acclimated animals). Two mortalities were observed in 21 C (70 F) acclimated animals which had moved up to the avoidance temperature (29 C or 84 F).

4.9.12 TEGULA FUNEBRALIS THERMAL PREFERENCE/AVOIDANCE

According to Morris et al. (1980), Tegula funebralis, the black turban snail, is common to very abundant in tidepools and on rocks along protected rocky California coastal areas in the middle intertidal region. Its geographical distribution extends from Vancouver Island, British Columbia, Canada to central Baja California, Mexico. It reaches a maximum shell diameter of 30 mm.

Tegula funebralis was not designated a representative important species (RIS) for the Diablo Canyon 316(a) demonstration but it is a common snail in the Diablo Canyon area. Because time and space were available, it was tested in the thermal preference/avoidance experiments.

The objective of the thermal preference/avoidance experiments was to provide information on the behavioral response of T. funebralis to a thermal gradient, specifically, whether it exhibits preference for or avoidance to a particular temperature. The purpose was to provide information about the possible response of the species to the thermal plume, in particular, whether sublethal temperatures would have a major effect on its distribution in Diablo Cove.

4.9.12.1 METHODS

A summary of the methods employed in the thermal preference/avoidance experiments on T. funebralis was presented in SECTION 4.5.1 of this report. A more detailed account of the experiment procedure used in this experiment is presented in APPENDIX B (Procedure 317). The protocols for this experiment are included in APPENDIX C of this report (experiments 17f to i).

Both juvenile (less than 15 mm shell diameter) and adult (greater than 15 mm diameter) specimens of T. funebralis were used in these runs. They were collected during early 1979 from the vicinity of Diablo Cove.

A total of four runs were made on this species. A summary of the run data is presented in TABLE 4.9.12-1. While being held in the laboratory prior to experimentation, the animals were fed to excess with Macrocystis and maintained in ambient temperature 4x8 ft flow-through tanks. During the holding period, the animals 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 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.

The 3-m gradient apparatus used for the experiment is described in APPENDIX A. The experimental procedure used was to establish the experimental temperature gradient in the experimental tank and to initiate the run by placing the test population in the compartment at the acclimation temperature for the test organisms. The organisms were then allowed to disperse in the tank for a period of hours without changing the temperature gradient. After at least one observation recording the position of the animals was made, the gradient was discontinued and the entire tank was maintained at the acclimation temperature of the organisms. Again after a period of hours, a final observation was made to verify that the animals could disperse throughout the tank. Groups animals

TABLE 4.9.12-1

PROTOCOL SUMMARY FOR TEGULA FUNEBRALIS
THERMAL PREFERANCE/AVOIDANCE

Run	Sample Size	Acclimation Temperature	Gradient Status (Temperature, C)		
			Initial	First Change	Second Change
17f[1]	30	ambient	8-30	13-14	--
17g[1]	30	18	7-31	--	--
17h[1]	30	18	8-37	18	--
17i[1]	31	21	7-37	21	--

[1] Run made with Haliotis cracherodii, Tegula brunnea and Haliotis rufescens also in the tank

acclimated to different temperatures were subjected to testing to determine the effects of acclimation on temperature avoidance.

It was possible to include more than one species simultaneously in the invertebrate thermal avoidance experiments, and this was done to the extent possible to increase efficiency. To be grouped together, species had to be compatible with each other (not mutual prey or predators) and of a size and mobility that precluded physical obstruction of movement in the tank.

4.9.12.2 RESULTS

A summary of the protocols for the runs on T. funebralis is presented in TABLE 4.9.12-1. The data are presented in the graphs shown in FIGURES 4.9.12-1 to 4.9.12-3.

Run 17f

The animals were introduced into the tank with a gradient already established (8-29 C). By 14.5 hours, the animals had dispersed over the entire tank (FIGURE 4.9.12-1). When the gradient was subsequently reduced to 13-14 C, by 38.5 hours the animals redistributed themselves over the portion of the tank that was previously excluded, and one large cluster formed at the end of the tank that had previously been the high temperature end.

Run 17g

In this run, 18 C-acclimated animals from the snail growth experiment were placed at their acclimation temperature within a range of 7-31 C. At 12 hours, they had dispersed over the entire tank.

Run 17h

This run involved a second test of the 18 C-acclimated animals. A 7-37 C gradient was established prior to placement of the animals in the tank. By 17.5

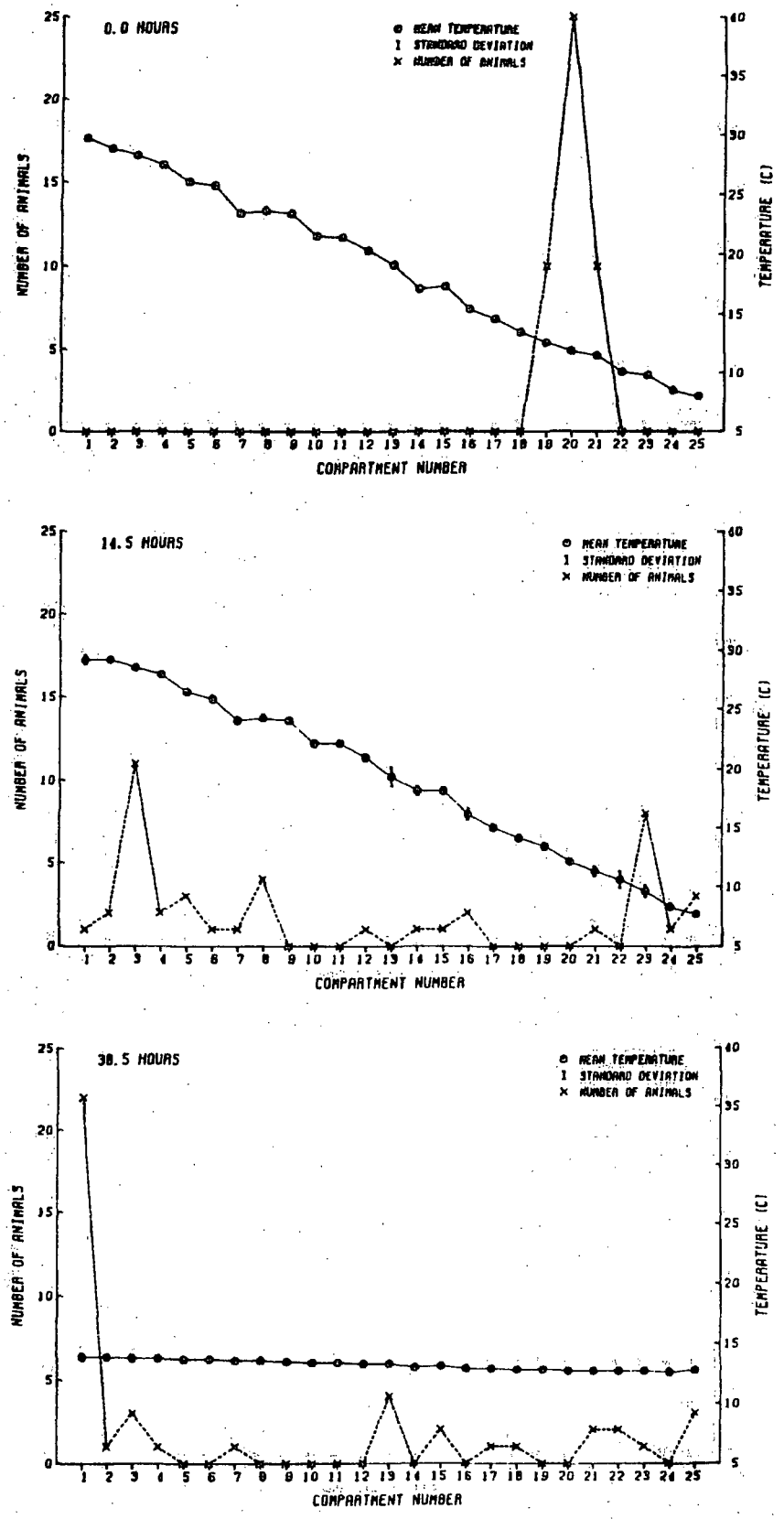


FIGURE 4.9.12-1

NUMBER OF ANIMALS AND TEMPERATURE VERSUS COMPARTMENT:
TEGULA FUNEBRALIS THERMAL PREFERENCE/AVOIDANCE
RUN 17F

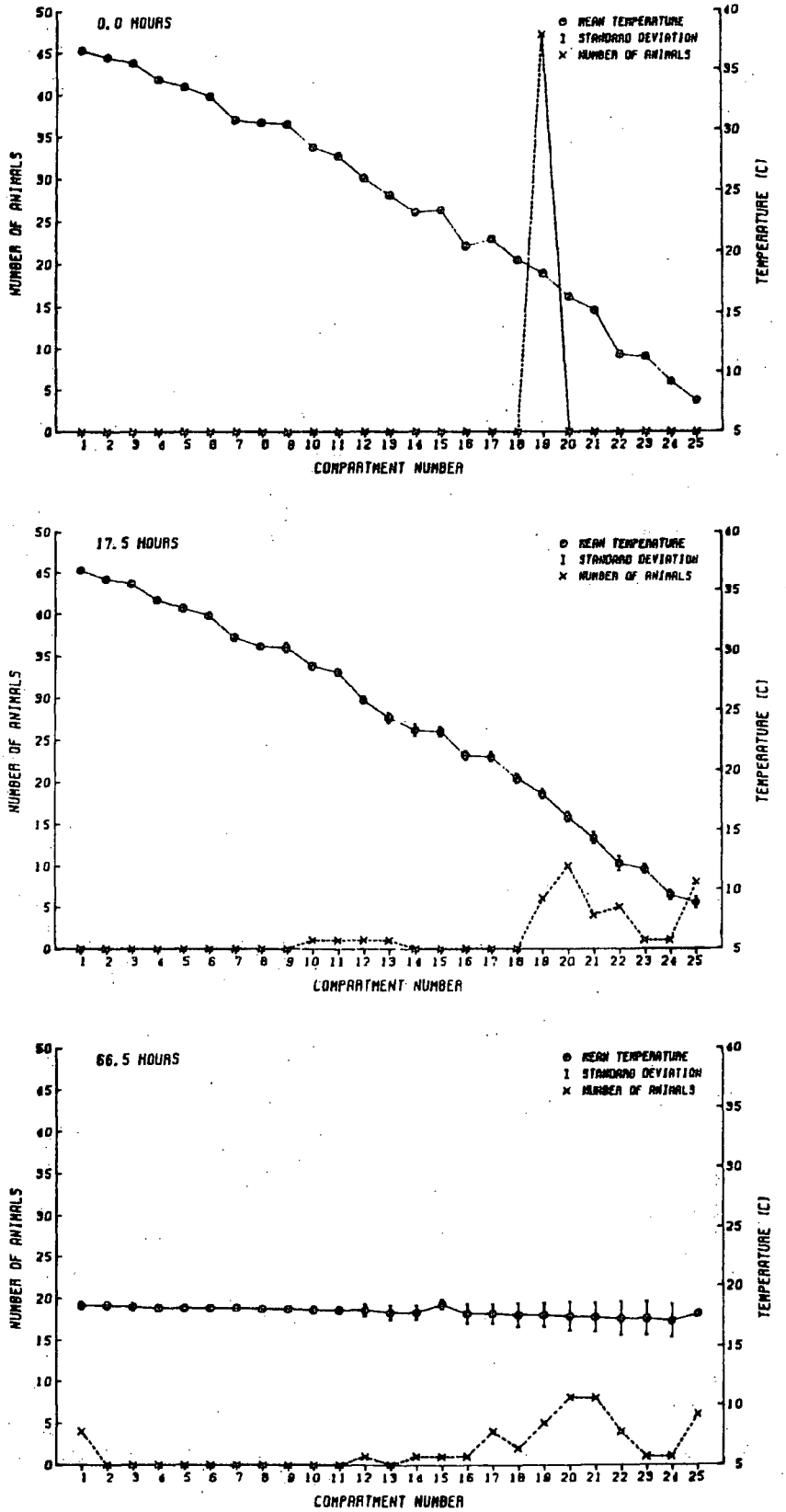


FIGURE 4.9.12-2

NUMBER OF ANIMALS AND TEMPERATURE VERSUS COMPARTMENT:
TEGULA FUNEBRALIS THERMAL PREFERENCE/AVOIDANCE
RUN 17H

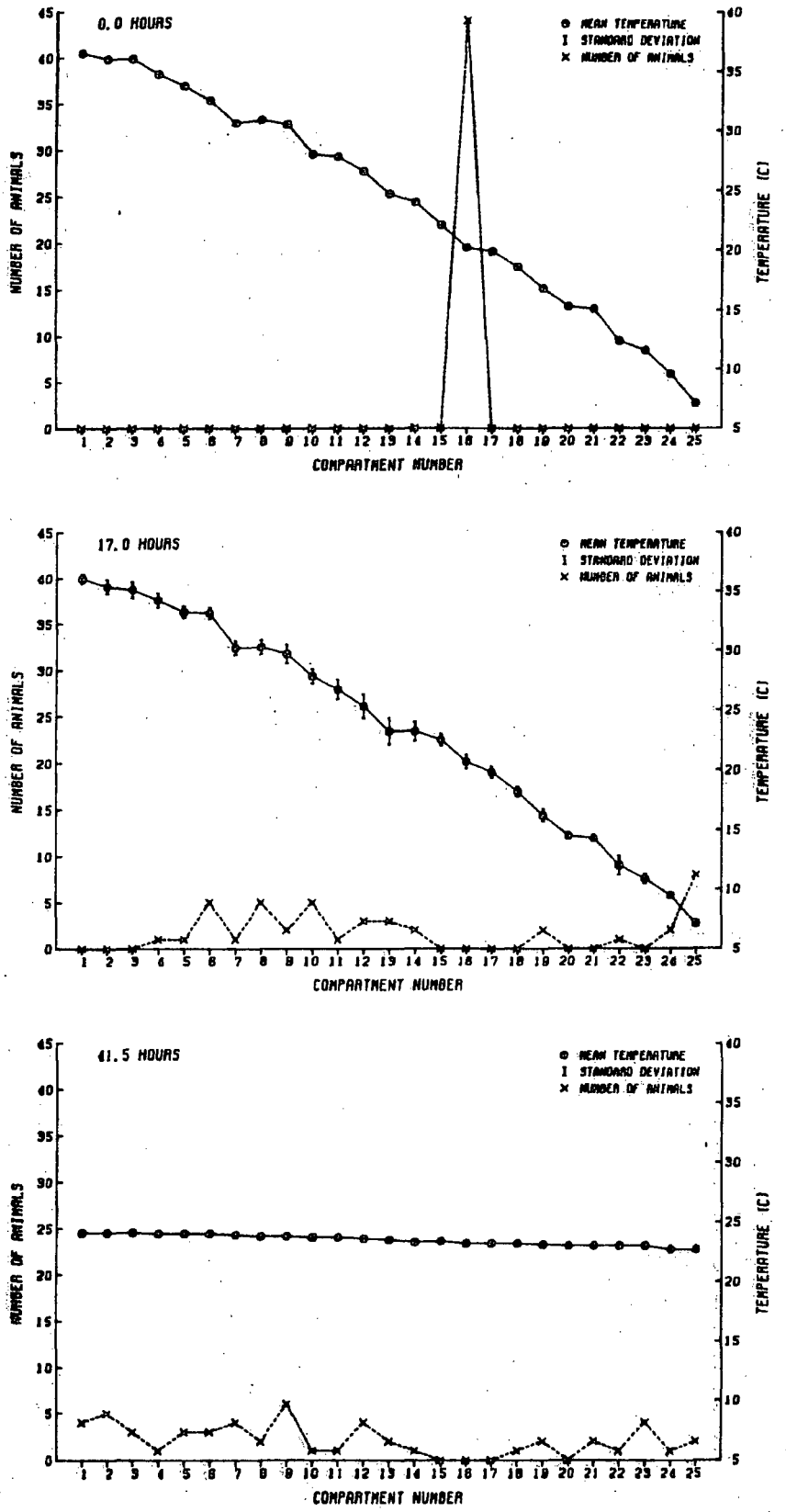


FIGURE 4.9.12-3

NUMBER OF ANIMALS AND TEMPERATURE VERSUS COMPARTMENT:
TEGULA FUNEBRALIS THERMAL PREFERENCE/AVOIDANCE
RUN 171

hours the animals had dispersed over the tank up to 27 C (FIGURE 4.9.12-2). The gradient was subsequently reduced to 17-18 C and by 66.5 hours a few animals had moved into the end compartment that was previously at the highest temperature.

Run 17i

In this final run on T. funebralis, 21 C-acclimated animals from the snail growth experiment were introduced into the tank with a 7-36 C gradient. At the 17 hour observation, they had dispersed across the tank up to 34 C (FIGURE 4.9.12-3). The gradient was subsequently reduced to 23-24 C and by 41.5 hours the animals had dispersed across the entire tank.

4.9.12.3 DISCUSSION

In general, the Tegula funebralis tested in this series of experiments did not exhibit a preference response to temperature. An avoidance response was consistently evident, however, in that the animals did not move into locations where the temperature exceeded a certain value. This "avoidance temperature" was positively related to the acclimation temperature of the animals prior to testing (about 27 C or 81 F for 18 C or 64 F acclimated animals and 34 C or 93 F for 21 C- or 70 F-acclimated animals).

5.0 LITERATURE CITED

Abbott, I. A. 1971. On the species of Iridaea (Rhodophyta) from the Pacific Coast of North America. Syesis 4:51-72.

Abbott, I. A. and G. Hollenberg. 1976. Marine algae of California. Stanford University Press. xii + 827 pp.

Abbott, D. P., R. B. Lawrence, and J. H. Phillips. 1964. An experiment in undergraduate teaching and research. Biological Sciences. Veliger 6: 1-6.

Adams, J. R. and D. G. Price. 1974. Thermal shock tolerances of larval red abalone (Haliotis rufescens). Environmental Investigations at Diablo Canyon, 1972-1973, PGandE, Dept. Engng. Res.

Arnon, D. I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Pl. Physiol., Lancaster, 24: 1-15.

Best, B. A. 1964. Feeding activities of Tegula funebralis (Mollusca: Gastropoda). Veliger 6: 42-46.

Brett, J. R. 1971. Energetic responses of salmon to temperature, A study of some thermal relations in the physiology and freshwater ecology of sockeye salmon (Oncorhynchus nerka). Am. Zool., 11:99-113.

Brett, J. R., J. E. Shelbourn and C. T. Schoop. 1969. Growth rate and body composition of fingerling sockeye salmon, Oncorhynchus nerka, in relation to temperature and ration size. J. Fish. Res. Bd. Can. 26:2363-2394.

Burge, R. T., and S. A. Schultz. 1973. The marine environment in the vicinity of Diablo Cove with special reference to abalones and bony fishes. Calif. Dept. Fish and Game, Mar. Res. Tech. Rpt. 19. 433 pp.

Clemens, W. A., and G. V. Wilby. 1961. Fishes of the Pacific Coast of Canada. Fish Res. Bd. Can. Bull. 68. 443 pp. 2nd ed.

Cox, K. 1962. California abalones, family Haliotidae. Calif. Fish and Game, Fish Bull., 118: 1-113.

Cox, K. W. 1960. Review of the abalone in California. Calif. Fish and Game 46: 381-406.

Crofts, O. R. 1937. The development of Haliotis tuberculata with special reference to organogenesis during torsion. Philos. Trans. R. Soc. Lond., Ser. B. Biol. Sc. 208: 219-268.

Curtner, W. W. 1917. Observation of the growth and habits of the red and black abalones. M.S. Thesis, Stanford Univ., 21 pp.

Darby, R. L., 1964. On growth and longevity in Tegula funebralis (Mollusca: Gastropoda). Veliger 6: 6-8.

Ebeling, Alfred W., and Richard N. Bray. 1976. Day versus night activity of reef fishes in a kelp forest off Santa Barbara, California. Fish. Bull. (U.S.) 74: 703-717.

Ebert, E. E. 1974. Red abalone temperature tolerance study. Pages 75-87 in Calif. Dept. Fish and Game, Diablo Canyon Power Plant Site Ecological Study Annual Report, July 1973 - June, 1974. State of Calif., Resources Agency, Dept. Fish and Game, Mar. Res. Adm. Rpt 74-10.

Ebert, E. E. 1974. Elevated temperature tolerance of the red abalone, Haliotis rufescens. Presented at a joint meeting of the American Fisheries Society, and the Wildlife Society, Monterey, California.

Ehrlich, K. F., J. M. Hood, G. Muszynski and G. E. McGowen. 1979a. Thermal behavioral responses of selected California littoral fishes. Fish. Bull. 76(4): 837-849.

Ehrlich, K. F., J. S. Stephens, G. Muszynski and J. M. Hood. 1979b. Thermal behavioral responses of the speckled sanddab, Citharichthys stigmaeus: laboratory and field investigations. Fish. Bull. 76(4): 867-872.

Farmanfarmaian, A. and A. C. Giese. Thermal tolerance and acclimation in the western purple sea urchin, Strongylocentrotus purpuratus. Physiol. Zool. 36237-243.

Feder, H. M. 1963. Gastropod defensive responses and their effectiveness in reducing predation by starfishes. Ecology 44(3): 505-512.

Feder, H.M., C. H. Turner and C. Limbaugh. 1974. Observations on fishes associated with kelp beds in southern California. Calif. Dept. Fish and Game, Fish Bull. 160. 144pp.

Fitch, J. E., and R. J. Lavenberg. 1971. Marine food and game fish of California. Univ. California Press, Berkeley. 179 pp.

Fleiss. 1973. Statistics for rates and proportions. Wiley and Co.

Frank, P. W. 1975. Latitudinal variation in the life history features of the black turban snail Tegula funebralis (Prosobranchia: Trochidae). Mar. Biol. 31: 181-192.

Frey, H. W. 1971. California's living marine resources and their utilization. State of Calif., Resources Agency, Dept. Fish and Game. 148 pp.

Fry, F. E. J. 1967. Responses of vertebrate poikilotherms to temperature. Pages 375-409 A. H. Rose, ed. Thermobiology. Academic Press, N.Y.

Galli, D. R. and A. C. Giese. 1959. Carbohydrate digestion in a herbivorous snail, Tegula funebralis. J. Exp. Zool. 140: 415-440.

Goddard, C. I., J. W. Lilley and J. S. Tait. 1974. Effects of M.S. 222 anesthetization on temperature selection in lake trout, Salvelinus namaycush. J. Fish Res. Board Can. 31(1): 100-103.

Gotshall, D. W. and L. Laurent. 1979. Pacific coast subtidal marine invertebrates. Sea Challengers, Los Osos, Calif. 107 pp. (+ maps).

Gotshall, D. W., L. L. Laurent, and F. E. Wendell. 1977. Diablo Canyon Nuclear Power Plant Site Ecological Study Annual Report, July 1, 1975 – June 30, 1976. State of Calif., Resources Agency, Dept. Fish and Game, Mar. Res. Admin. Rpt. 77-18 and Quarterly Rpt. No. 12. April 1, 1976 – June 30, 1976.

Hall, L. W., C. H. Hocutt and J. R. Stauffer. 1978. Implication of geographic location on temperature preference of white perch, Morone americana. J. Fish. Res. Board Can. 35: 1464-1468.

Hansen, J. E. 1977. Ecology and natural history of Iridaea cordata (Gigartinales, Rhodophyta) growth. J. Phycol. 18: 395-402.

Hart, J. L. 1973. Pacific fishes of Canada. Fish. Res. Bd. Can. Bull. 180. 740 pp.

Hill, L. G., G. D. Schnell and J. Pigg. 1975. Thermal acclimation and temperature selection in sunfishes (Lepomis, Centrarchidae). Southwestern Nat. 20(2): 177-184.

Howorth, P. 1977. Foraging along the California coast. Capra Press and Ross/Erikson, Santa Barbara. 192 pp.

Hsiao, S. and L. Druehl. 1971. Environmental control of gametogenesis in Laminaria saccharina. I. The effects of light and culture media. Can. J. Bot. 49(8): 1503-1508.

Hunt, D. E. 1977. Population dynamics of Tegula and Calliostoma in Carmel Bay, with special reference to kelp harvesting. M.S. Thesis. San Francisco State University. 70 pp.

Icanberry, J. W., J. W. Warrick, and D. W. Rice, Jr. 1978. Seasonal larval fish abundance in water off Diablo Canyon, California. Trans. Am. Fish. Soc. 107(2): 225-233.

Ino, T. 1952. Biological studies on propagation of Japanese abalone (genus Haliotis). Bull. Tokai Reg. Fish. Res. Lab. 5. 102 pp.

Javaid, M. Y., and J. M. Anderson. 1967a. Thermal acclimation and temperature selection in Atlantic salmon, Salmo salar, and rainbow trout, S. gairdneri. J. Fish. Res. Board Can. 24(7): 1507-1513.

Javaid, M. Y., and J. M. Anderson. 1967b. Influence of starvation on selected temperature of some salmonids. J. Fish. Res. Board Can. 24(7): 1515-1519.

Johansen, H. W., and L. F. Austin. 1970. Growth rates in the articulated coralline Calliarthron (Rhodophyta). Can. J. Bot(1): 125-132.

Kwain, W. H., and R. W. McCauley. 1978. Effects of age and overhead illumination on temperatures preferred by underyearlying rainbow trout, Salmo gairdneri, in a vertical temperature gradient. J. Fish. Res. Board Can. 35: 1430-1433.

Leighton, D. L. 1972. Laboratory observations on the early growth of the abalone, Haliotis sorenseni, and the effect of temperature on larval development and settling success. Fish. Bull. (U.S.) 70: 373-381.

Leighton, D. L. 1964. Grazing, Pages 71-98 in An investigation of the effects of discharged wastes on kelp. Calif. State Water Quality Bd. Publ. 26.

Leighton, D. L. 1966. Studies of food preferences in algivorous invertebrates of southern California kelp beds. Pacific Science 10(1): 104-113.

Leighton, D. L. 1968. A comparative study of food selection and nutrition in the abalone, Haliotis rufescens (Swainson) and the sea urchin, Strongylocentrotus purpuratus (Stimpson). Ph.D. Thesis, Univ. of Calif., San Diego 213 pp.

Leighton, D. L. 1974. The influence of temperature on larval and juvenile growth in three species of Southern California abalone. Fish. Bull. (U.S.) 72(4): 1137-1145.

Leighton, D. L., and R. A. Boolootian. 1963. Diet and growth in the black abalone, Haliotis cracherodii. Ecology 44: 227-238.

Limbaugh, C. 1955. Fish life in the kelp beds and the effect of kelp harvesting. Univ. Calif. Inst. Mar. Sc., IMR Ref. 55-9. 158 pp.

Love, M. S. 1974. Food habits of three midwater kelp-bed predators. M.A. Thesis. Univ. Calif., Santa Barbara. 50 pp.

Lowry, L. G., A. J. McElroy and J. S. Pearse. 1974. The distribution of six species of gastropod molluscs in a California kelp forest. Biol. Bull. 147(2): 386-396.

Lünning, K., and M. J. Dring. 1975. Reproduction, growth, and photosynthesis of gametophytes of Laminaria saccharina. Mar. Biol. 29:295-200.

Lünning, K., and M. Neushul. 1978. Light and temperature demands for growth and reproduction of laminarian gametophytes in southern and central California. Mar. Biol. 45: 297-309.

McBeth, J. W. 1972. The growth and survival of the California red abalone in Japan. Venus 31: 122-126.

McCauley, R. W. 1970. Preferred temperature of yearling lake trout, Salvelinus namaycush. J. Fish. Res. Board Can. 27: 1729-1733.

McCauley, R. W., and N. W. Huggins. 1979. Ontogenetic and non-thermal seasonal effects on thermal preferenda of fish. Amer. Zool. 19: 267-271.

McCauley, R. W. and L. A. A. Read. 1973. Temperature selection by juvenile and adult yellow perch (Perca flavescens) acclimated to 24 C. J. Fish Res. Board Can. 30: 1253-1255.

McLean, J. H. 1966. West American Prosobranch Gastropoda: superfamilies Patellacea, Pleurotomariacea and Fissurellacea. Ph.D. Thesis Stanford Univ., 262 pp.

Medvick, P.A., and J. M. Miller. 1979. Behavioral thermoregulation in three Hawaiian reef fishes. Env. Biol. Fish. (4(1): 23-28.

Miller, D. J., and J. J. Geibel. 1973. Summary of blue rockfish and lingcod life histories; a reef ecology study; and giant kelp, Macrocystis pyrifera, experiments in Monterey Bay, California. Calif. Dept. Fish and Game, Fish Bull. 158. 137 pp.

Miller, D. J., Odemar and D. W. Gotshall. 1967. Life history and catch analysis of the blue rockfish (Sebastodes mystinus) off central California, 1961-1965. State of Calif., Resources Agency, Dept. Fish and Game, Mar. Res. Adm. Rpt. 67-14.

Miller, D. J., and J. E. Hardwick. 1973. The status of the rockfish resource and its management. State of Calif., Resources Agency, Dept. Fish and Game, Mar. Res. Tech. Rpt. 17.

Miller, D. J., and R. N. Lea. 1972. Guide to coastal marine fishes of California. Calif. Dept. Fish and Game, Fish Bull. 157. 235 pp.

Morris, R. H., D. P. Abbott and E. C. Haderlie. 1980. Intertidal Invertebrates of California. Stanford Press. 690 pp.

Morse, D. E., H. Duncan, N. Hooker, and A. Morse. 1977. Hydrogen peroxide induces spawning in mollusks, with activation of prostaglandin endoperoxide synthetase. Science 196: 298-300.

Morse, D. E., N. Hooker, H. Duncan, and L. Jensen. 1979a. Gamma-aminobutyric acid, a neurotransmitter, induces planktonic abalone larvae to settle and begin metamorphosis. Science 204(27): 407-410.

Morse, D. E., N. Hooker, L. Jensen, and H. Duncan. 1979b. Induction of larval abalone settling and metamorphosis by gamma-aminobutyric acid and its congeners from crustose red algae: applications to cultivation, seed production and bioassays; principal causes of mortality and interference. Proc. World Mariculture Soc. 1979. 23 pp.

Murayama, S. 1935. On the development of the Japanese abalone, Haliotis gigantea. J. Coll. Agric. Tokyo Imp. Univ. 13: 227-233.

Norris, K. S. 1963. The functions of temperature in the ecology of the percoid fish Girella nigricans (Ayres). Ecol. Monogr. 33: 23-62.

North, W. J., and M. B. Schaefer. 1964. An investigation of the effects of discharged wastes on kelp. Calif. State Water Quality Control Board Publ. No. 26. 124 pp.

North, W. J. 1966. An evaluation of the marine fauna and flora in the vicinity of Diablo Cove, California. Marine Advisors, La Jolla, Calif., 38 pp.

Oba, T. 1964. Studies on the propagation of an abalone, Haliotis diversicolor supertexta Lischke-II. On the development. Bull. Jap. Soc. Sc. Fish. 30: 809-819.

O'Connell, Charles P. 1953. The life history of the cabezon Scorpaenichthys marmoratus (Ayres). Calif. Dept. Fish and Game, Fish. Bull. 93. 76 pp.

Olsen, D. A. 1971. The potential for an abalone fishery in Hawaii. Ph.D Thesis, Univ. of Hawaii. 346 pp.

Paine, R. T. 1969. The Pisaster-Tegula interaction: prey patches, predator food preference, and intertidal community structure. Ecology 50: 950-961.

Paine, R. T. 1971. Energy flow in a natural population of the herbivorous gastropod Tegula funebralis. Limn. Ocean. 16: 86-98.

Paine, R. T., and R. L. Vadas. 1969. Calorific value of benthic marine algae and their postulated relation to invertebrate food preferences. Mar. Biol. 4(2): 79-86.

Paul, A. J., J. M. Paul, D. W. Hood and R. A. Neve. 1977. Observations on food preferences, daily ration requirements and growth of Haliotis kamschatkana Jonas in captivity. Veliger. 19(3): 303-309.

Peppard, B. C. 1964. Shell growth and repair in the Gastropod Tegula funebralis. Veliger 6: 59-63.

PGandE. 1977. Diablo Canyon Nuclear Power Plant 316(a) Demonstration. Nine month Progress Report. October 1977.

PGandE. 1978. Diablo Canyon Power Plant 316(a) Demonstration. Nine month Progress Report. May 1978.

PGandE. 1979a. Diablo Canyon Power Plant 316(a) Demonstration. Nine month Progress Report. February 1979.

PGandE. 1979b. Diablo Canyon Power Plant 316(a) Demonstration. Nine month Progress Report. November 1979.

PGandE. 1980. Diablo Canyon Power Plant 316(a) Demonstration. Nine month Progress Report. August, 1980.

Poore, G. C. B. 1972. Ecology of New Zealand abalones, Haliotis species (Mollusca: Gastropda). I. Feeding. N. Z. Jour. Marine FW Res. 61(1-2): 11-12.

Prosser, C. L. 1973. Comparative Animal Physiology. 3rd Ed. W. B. Saunders Co. Phil. 966 pp.

Reynolds, W. W., and M. E. Casterlin. 1979. Behavioral thermoregulation and the "Final preferendum" paradigm. Amer. Zool. 19: 211-224.

Richards, F. P. 1978. The preferred temperatures of the brown bullhead, Ictalurus nebulosus, with reference to its orientation to the discharge canal of a nuclear power plant. Trans. Amer. Fish. Soc. 107(2): 288-294.

Ricketts, E. F., J. Calvin and J. W. Hedgepeth. 1968. Between Pacific tides. Stanford Univ. Press, Stanford, Calif. 614 pp. 4th ed.

Roed, K. H. 1979. The temperature preference of the three-spined stickleback, Gasterosteus aculeatus L. (Pisces), collected at different seasons. Sarsia 64:137-141.

Roedel, P. M. 1953. Common ocean fishes of the California coast. Dept. Fish and Game, Fish Bull. 91. 184 pp.

Sokal, R. and J. Rohlf. 1969. Biometry. W. H. Freeman and Co., San Francisco. 776 pp.

Shepherd, S. A. 1973. Studies on southern Australian abalone (Genus Haliotis). I. Ecology of five sympatric species. Aust. Jour. Marine FW Res. 24: 217-257.

Standard Methods for the Examination of Water and Wastewater. 1976. pp. 733-743. Analysing results of quantal bioassays. American Public Health Association, Washington, D. C. 14th ed.

Steffel, S., A. E. Dizon, J. J. Magnuson and W. H. Neill. 1976. Temperature discrimination by captive free-swimming tuna, Euthynnus affinis. Trans. Amer. Fish. Soc. 5:588-591.

Sullivan, C. M. and K. C. Fisher. 1953. Seasonal fluctuations in the selected temperature of speckled trout, Salvelinus fontinalis (Mitchill). J. Fish. Res. Board Can. 10(4):187-195.

Sullivan, C. M. and K. C. Fisher. 1954. The effects of light on temperature selection in speckled trout, Salvelinus fontinalis (Mitchill). Biol. Bull. 107:278-288.

TERA. 1982. Thermal Discharge Assessment Report Diablo Canyon Power Plant, Pacific Gas and Electric. TERA Corporation.

Terry, C. B. and J. S. Stephens. 1976. A study of the orientation of selected embiotocid fishes to depth and shifting seasonal vertical temperature gradients. Bull. So. Calif. Acad. Sci. 75(2):170-183.

Vadas, R. 1972. Ecological implications of culture studies on Nereocystis luetkeana. J. Phycol. 8:196-203.

Van Olst, J. C., J. M. Carlberg and D. Lapota. 1979. Growth - Temperature - Survival relationships in abalone. Hubbs/Sea World Research Inst. Tec. Rep., 79-116. 54 pp.

Waaland, J. R. 1973. Experimental studies in the marine algae Iridaea and Gigartina. J. Exp. Mar. Biol. Ecol. 11:71-80.

Wara, W. A. and B. B. Wright. 1964. The distribution and movement of Tegula funebralis in the intertidal region, Monterey Bay, California (Mollusca: Gastropoda). Veliger 6:30-38.

Wright, M. B. 1975. Growth in the black abalone Haliotis cracherodii. Veliger 18:194-199.