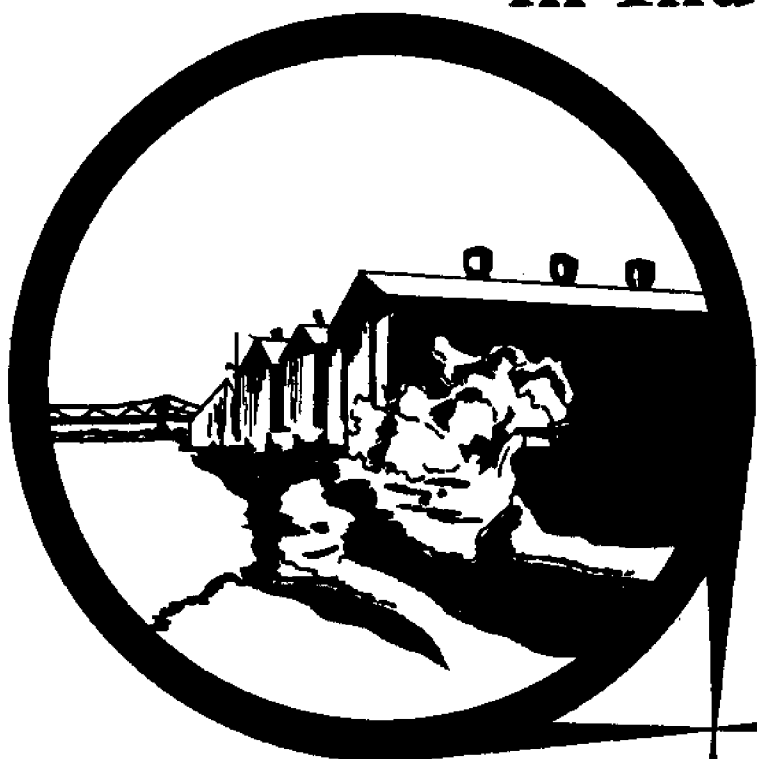
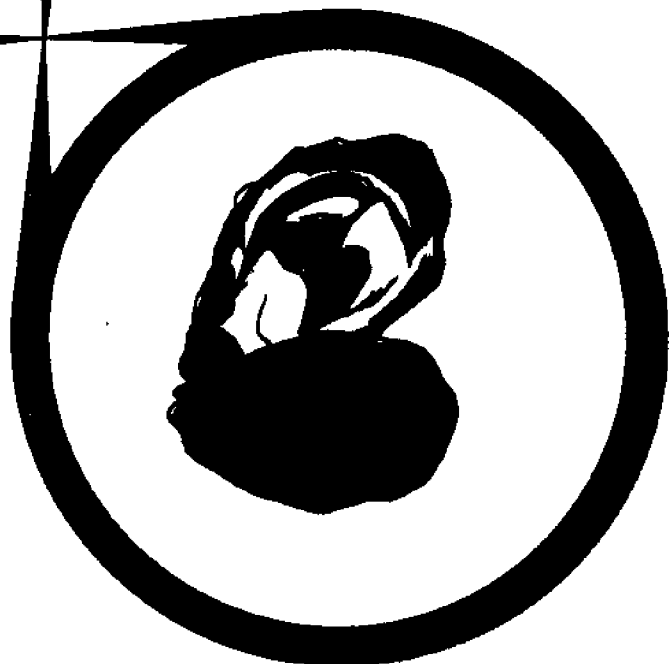


# The Effects of Thermal Effluent on the American Oyster *Crassostrea virginica* Gmelin in Indian River Bay, Delaware



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& Don Maurer



THE EFFECTS of THERMAL EFFLUENT on the AMERICAN OYSTER,  
CRASSOSTREA VIRGINICA GMELIN,  
in INDIAN RIVER BAY, DELAWARE

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Submitted to:

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## ABSTRACT

In June 1970, 2025 oysters (Crassostrea virginica) were divided among three stations in Indian River Bay (Lat. 38° 35'; W. Long. 75° 14'): 1) at the intake of the Delmarva Power and Light Co., 2) 2.5 km downstream at the mouth of the effluent canal, and 3) at a station 6 km east of the plant at Oak Orchard. High mortalities necessitated placement of 6600 additional oysters in July at the same three stations. These were sampled from August 1970 to May 1971.

The heated effluent had the effect of shortening and modifying the severity of the winter, thereby increasing the growing season. Oysters in the effluent showed the greatest shell growth throughout the study. Meat weights and glycogen concentration in the effluent were highest of all the stations in the winter. Summer conditions in the effluent were severe due to excessively high temperatures and meat weights and glycogen concentrations were lower than at other stations. In the spring, spawning may have occurred prematurely in the effluent at Station Two. The study showed that the thermal effluent has both beneficial and detrimental effects depending on the season.

## INTRODUCTION

This research was undertaken in order to determine some of the effects of the thermal discharge from the Delmarva Power and Light Company plant at Millsboro, Delaware, on the American oyster, Crassostrea virginica (Gmelin), placed in Indian River Bay (Fig. 1). The objectives of the project were to trace the survivorship, growth, condition, and reproductive behavior of oysters held in the thermal effluent and at control stations.

Fossil-fuel power plants are about 40% efficient in converting the energy of coal to electrical energy (Sorge, 1969). The remaining 60% is given off as waste heat energy. Most of this energy must be removed by the local water supply which is pumped through heat exchangers in the steam condensers of the power plant. Thermal addition involves the non-consumptive use of water for cooling purposes producing an effluent often significantly warmer than the ambient water. Because this waste heat may have a wide variety of effects upon the local biota, thermal addition has become a cause for concern.

Mihursky et al., 1970, defined thermal pollution as industrially induced, unnatural temperature changes which

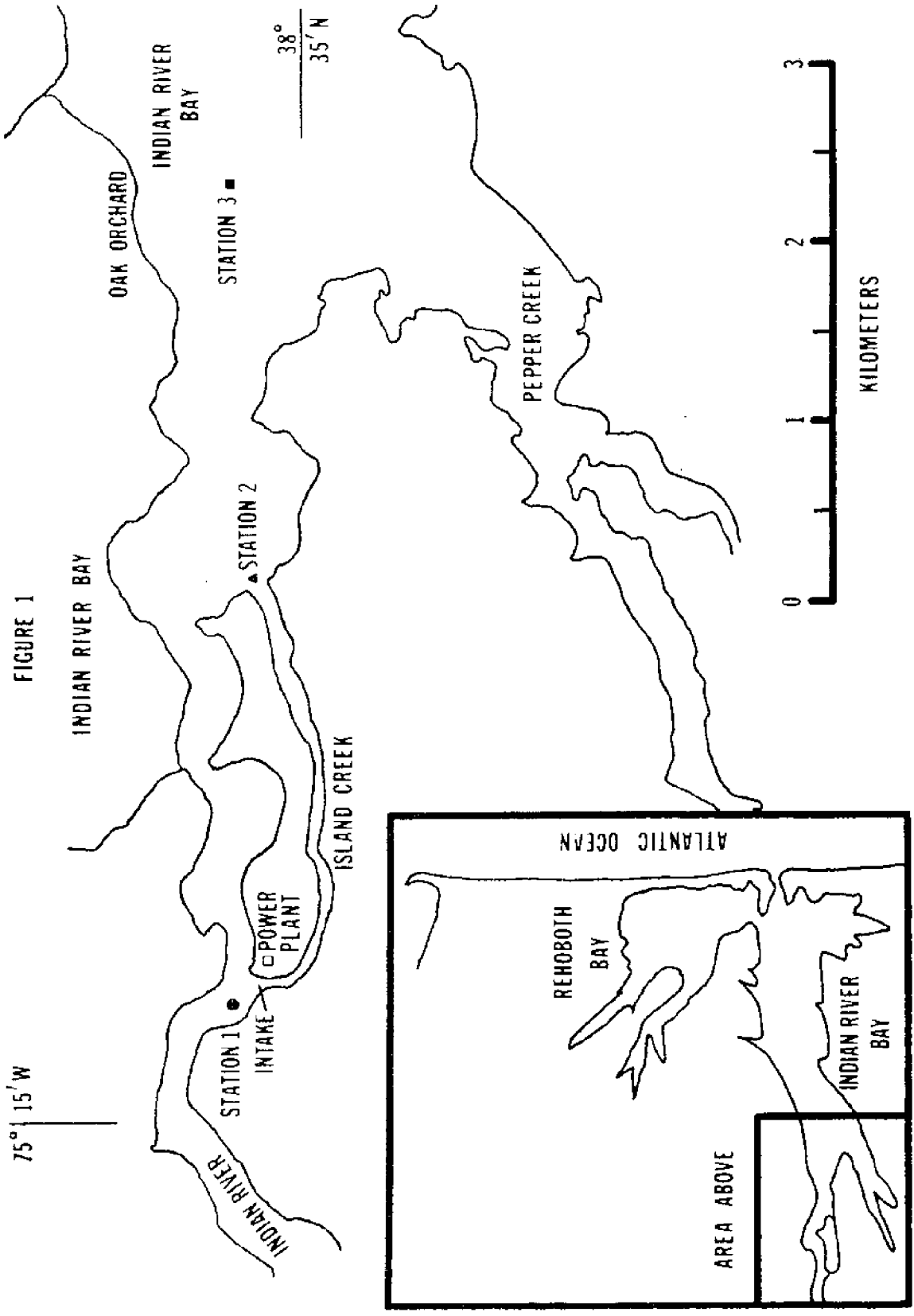


FIGURE 1

cause alterations in an aquatic system to the extent that other legitimate uses are impaired. In addition to numerous field and laboratory studies, many general articles have been published dealing with the local, regional, and national problems of waste heat disposal: Anonymous, 1956; Markowski, 1959; Davidson and Bradshaw, 1967; Vernberg, 1967; Cairns, 1968; Cronin, 1968; Mihursky, 1968; Singer, 1968; Clark, 1969; Cole, 1969; Davis, 1969; Nash, 1969; North and Adams, 1969; Sorge, 1969; Zieman, 1969; Arnold, 1970; Gilluly, 1970; Holcomb, 1979; Cairns, 1971; Ingle et al., 1971; Wei, 1971; Levin et al., 1972.

When waste heat is eliminated in a one-pass system and water is returned to a lake, river, or estuary at a higher than ambient temperature, a thermal effluent is created. In this presentation, effluent, thermal effluent, heated effluent, effluent area, and thermal addition will be used synonymously. The term "thermal disturbance" is applied to the total change made in the water by the power plant including temperature, biocides, trace metals, suspended sediment, current velocity, turbulence, dissolved oxygen, and the effect of this altered water on other biota associated with the oysters.

Clark (1969) stated that in 1968 the electric power industry was responsible for 75% of the 60,000 billion gallons of cooling water used in this country. The projected future cooling needs of the electric power industry



vary greatly. Clark (1969) estimated that by the end of the century cooling water needs will reach one-third of the average daily freshwater run-off of the country. The estimates of Sorge (1969) indicate that we will use twice our average daily freshwater run-off by the year 2000. Many river systems receive the majority of their annual run-off during the spring months and least run-off during the late summer. Therefore, the season of highest ambient temperature coincides with the time when the greatest percentage of the daily stream flow must be used for cooling. For this reason, there has been an increased tendency to locate power plants adjacent to estuaries. Because of tidal flushing with cool ocean water, these power plants would no longer be totally dependent on run-off. A nuclear power plant on Chesapeake Bay at Chalk Point, Maryland uses 500,000 gallons of cooling water per minute which often exceeds the freshwater flow passing this point on the estuary (Davidson and Bradshaw, 1967). By 1980, nearly one-third of all power plants in this country will use estuarine water for cooling purposes (Picton, 1960), and it seems likely that the trend will continue. It appears certain that alternate cooling methods or alternate energy sources must be developed or our demands on estuarine waters may cause dramatic changes in these highly productive areas.

There are many factors which make the oyster a suitable assay organism for thermal addition studies. The oyster family (Ostreidae) has a long geologic history as a family and was commonly associated with estuaries in the past. Today they are abundant in estuaries along our entire Atlantic and Gulf Coasts where they are exposed to greatly differing temperature regimes. This makes them a truly representative estuarine species. Some populations occur intertidally where they survive wide temperature fluctuations. Although the oyster may tolerate a wide variety of thermal conditions, temperature controls the rate of virtually all of its life functions. Compared with older oysters, young oysters show rapid growth, maximizing differences in the growth rate. This difference can be exploited as a sensitive measure of change in thermal studies (Maurer, unpublished data). Oysters also exhibit a seasonal glycogen-gonad cycle which is temperature-dependent. They are relatively easy to collect, clean, and sample. They feed on suspended unicellular algae and can be successfully held in containers provided they are exposed to a sufficient flow of water. Their sedentary nature as adults also makes them particularly susceptible to the effects of heated effluents. Finally, the oyster was selected because of the abundant literature available on the biology of this species.

Prior to 1957, seed oysters were planted and marketable oysters were harvested annually in Indian River and Rehoboth Bays (Humphries and Daiber, 1968). In that year, the protozoan parasite, Minchinia nelsoni (Haskin, Stauber, Mackin) ("MSX"), reached epizootic proportions, causing excessive mortalities in many oyster populations on the Atlantic Coast (Zimmerman and Rosenfield, 1967; Andrews, 1968). During this period, the natural beds of Delaware Bay were decimated and planted beds in Indian River and Rehoboth Bays were virtually eliminated. During the past decade, attempts to raise MSX-resistant stocks have been made by several researchers (Haskin, 1965; Andrews, 1968; Maurer, 1970). Since the incidence of MSX has declined and some of the natural beds in Delaware Bay are becoming re-established, it may be that the surviving, natural stock of oysters are beginning to develop some disease resistance, either genetic or acquired. From these "resistant" stocks of oysters, commercial planting of oysters may again become feasible in suitable small bays.

The massive amount of literature on the biological effects of temperature and thermal effluents has been summarized in various bibliographies (Kennedy and Mihursky, 1967; Nakatani et al., 1968; Templeton et al., 1969; Coutant, 1970; Coutant et al., 1972; Raney and Menzel, 1969;

Jensen et al., 1969; and others). Those references most pertinent to oysters and shellfish research in general will be mentioned.

The temperature regime affects the life of the oyster in many ways: controlling the rate of water transport, respiration, feeding, utilization of food reserves, gonadal development, and time of spawning. The American oyster is normally found in water between 1-36°C, but little is known about prolonged effects above 32°C (Galtsoff, 1964). Dunnington (1968) found that oysters survived longer at lower temperatures when held under anaerobic conditions. This is probably due to the lower metabolic rate at lower temperatures which allows conservation of stored food reserves. Mackin (1961) agreed that extremes of the physical environment such as salinity and temperature may be one factor leading to increased oyster mortalities.

Because it affects feeding rate, metabolic rate, and conversion efficiency, thermal addition causes changes in the growth rate of the oyster. Clark (1969) suggests that thermal effluents could be of use in mariculture to maximize the growth of fish. Barnett and Hardy (1969), in studying the snail, Nassarius reticulata (L.), found that individuals within the effluent area developed thinner shells than those found outside it. This may be due to

the higher metabolic demand in the heated water leaving less energy available for shell growth. Kennedy et al. (1969) found differences in shell components and configuration in bivalves associated with elevated temperatures. Ryther and Bardach (1968) report remarkable growth for oysters held in a thermal effluent in Long Island. The hard clam, Mercenaria mercenaria (L.) was found to grow throughout the year in its southern range, but only seasonally in its northern range (Ansell, 1968). Butler (1965), however, found increased meat weights but no additional shell growth in oysters held in an effluent. This is possible despite increased metabolic demands because many of the suitable algal food populations are at high levels at temperatures above 30°C (Davis and Calabrese, 1964).

One means of determining the condition of oysters is to measure the glycogen concentration. Glycogen is animal starch and is the main food reserve of the oyster. An oyster high in glycogen is said to be "fat" and in good condition. The application of the term "fat" by shellfisheries' biologists to oysters high in glycogen is a misnomer reflecting the condition of being high in storage products, which is not biochemically correct. Glycogen concentration varies seasonally in oyster populations (Galtsoff, 1964). Ansell (1968) recorded a loss

of fatness in hard clams held in a thermal effluent. Oysters are also known to lose fatness at elevated temperatures (Medcof, 1946). Chipman (1948) commented that loss of fatness at elevated temperatures was associated with the more rapid conversion of glycogen to glucose due to higher metabolic demands.

It is relatively well established that oyster gonadal development and spawning are triggered by environmental parameters and that the most important of these is temperature (Nelson, 1928). The annual cycle of gonad development in the oyster has been well documented by several researchers (Loosanoff, 1942; Kennedy and Battle, 1964). Oysters held in the effluent would be exposed to temperatures favoring gonad development and spawning before those at control stations (Loosanoff and Davis, 1963; Galtsoff, 1964). For this reason, attempts were made to spawn individuals in the lab during the early spring months using the methods of Loosanoff and Davis (1963), Maurer and Price (1968), and Price and Maurer (1971). It seems likely that premature spawning would be exposed to unfavorably cold water outside of the effluent area.

Indian River Bay has a total area of 9,555 acres (Humphries and Daiber, 1968). In the past, it was an important oyster growing area, but today the main com-

mercial species is the hard clam, Mercenaria mercenaria. The Delmarva Power and Light Company plant on Burton's Island has a maximum operating capacity of 350 megawatts (mw) and uses 265,000 gallons of cooling water per minute. This accounts for about one-tenth of the mean freshwater flow for cooling purposes. In addition, average tidal fluctuations make an additional 20,000 cu. ft./sec. available for plant use. Island Creek acts as a natural discharge canal carrying the heated effluent water 2.5 km downstream before it rejoins Indian River Bay. Based on a mean tidal excursion of 1.2 km, the recirculation of effluent water is unlikely as the distance from the mouth of Island Creek to the intake is about 3.0 km (Gibbons and Brady, 1971). Indian River Bay is relatively shallow near the power plant and water temperatures follow air temperatures closely. For this reason, rather large diurnal temperature fluctuations are not uncommon. During monthly collecting trips ambient temperature ranges were 2-30°C throughout the year. The effluent temperature range was 8-40°C. An all night hydrographic study in the effluent may remain above 35°C throughout a twenty-four hour period.

## MATERIALS and METHODS

Following a two-year pilot study conducted by our laboratory during 1968-1970 (Maurer, unpublished), a more extensive twelve-month study was begun in June 1970. During the spring of 1970, the necessary field equipment was constructed. Three stations were selected in Indian River Bay, Delaware (Fig. 1). Station One, one hundred meters upstream from the cooling water intake of the power plant, and Station Three, six kilometers east of the plant at Oak Orchard, were chosen as control stations. Station Two was placed at the mouth of Island Creek, 2.5 km east of the plant. Island Creek serves as the effluent canal of the power plant. Spot checking showed that the amount of heat dissipated from Island Creek in transit from the plant discharge to the mouth of Island Creek was small, especially in summer. Each station was marked by an anchored wooden tripod bearing a sign identifying the project and warning pleasure boats to avoid the immediate area. Nine trays were constructed during the spring of 1970. These consisted of an iron frame (90 x 45 x 16.5 cm) supported on legs 60 cm in length. Small iron plates near the base of each leg served to prevent the trays from sink-



ing into the soft mud. Each tray contained a rectangular basket of half-inch welded wire which held the oysters. A buoy line was tied to a rope harness on each tray marking its position and making monthly recovery possible.

In early June 1970 oysters were collected by hand from the intertidal oyster bars of the Murderkill River, Bower's Beach, Delaware. Oysters were culled in the laboratory. This involved removing oyster spat and fouling organisms and reducing the clusters of oysters to individuals. The largest oysters were discarded and the smaller oysters (4-9 cm) were selected as most appropriate for the study. Younger individuals tend to grow faster (Maurer, unpublished data), thus maximizing any differences in growth due to the experimental differences in temperature.

On June 10, 1970, 2025 oysters were placed at the three stations. These were called Group One oysters. Two hundred and twenty-five oysters were placed in each of the nine sea racks which were distributed with three racks at each of the three stations. High mortalities during June necessitated the collection of a second group of oysters also from the Murderkill River. The remaining Group One oysters were consolidated into two trays per station. Nine additional trays were constructed. The oysters were culled as before and six thousand six hundred were placed at the three stations on July 24, 1970 in four trays of

five hundred and fifty at each station. These were called Group Two oysters. This provided a sufficiently large number of oysters so that expected mortalities and sampling over the twelve month period would not reduce the number of oysters to levels insufficient for sampling and analysis.

During the summer months (June-August) the trays were examined (two to four times each month) and silt and fouling organisms were removed to minimize mortality and reduce the limitations on growth due to these factors. For the remainder of the year (September-May) monthly cleaning was sufficient to control fouling organisms. All oysters were counted on monthly field trips to determine mortality. Samples of oysters were collected randomly from each group at each station and returned to the laboratory for study. Water temperature, salinity, and dissolved oxygen were measured during field trips using a Hydrolab, Model II A. This instrument was calibrated using a laboratory thermometer, a Bissett Berman portable laboratory salinometer, Model 6230, and the Winkler titration method of determining dissolved oxygen.

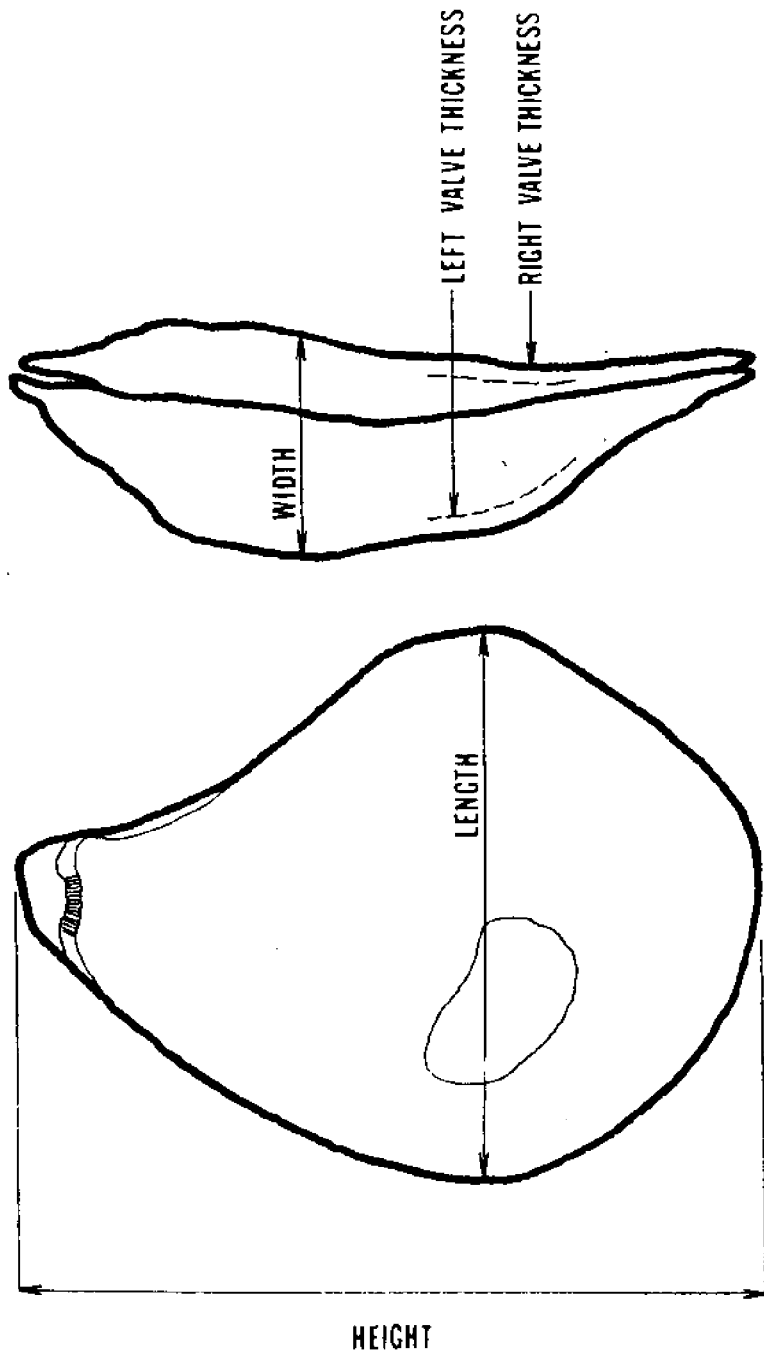
These data were supplemented by the extensive continuous temperature data collected by the Delmarva Power and Light Company. A spring drive Foxboro seven-day continuous temperature recorder was used for this purpose.

Derickson (1970) also presents hydrographic data taken in the Indian River Bay area.

One hundred oysters were retained at the laboratory and measured to provide a baseline of size and condition of oysters at the beginning of the study and for comparison with oysters from the three study stations. Shell height, length, and width were determined to the nearest millimeter with a field measuring board. Shell thickness was measured to the nearest tenth of a millimeter with a micrometer caliper (Fig. 2). Because of random variation and breakage during handling, this method of determining shell dimensions may contribute to so-called negative shell growth. Therefore, one hundred oysters were set aside at each station and these individuals were measured during each monthly collection from January through May. When mortalities occurred in this group, they were replaced by living individuals of the same size.

Oysters used for meat weight determinations were opened carefully with minimal physical damage to the oyster tissues. They were blotted and wet weights were determined using a Mettler Balance, Model H 20 T. They were then oven dried to constant weight at 80°C. Constant weight was determined by weighing several oysters on several consecutive days. Dry weights of the meat were then taken after cooling in a dessicator jar. Meat

FIGURE 2  
OYSTER SHELL DIMENSIONS



weights provide an indication of meat growth, and percent water values can be determined using wet and dry weights. Percent water is an indication of oyster condition.

Another measure of oyster condition is glycogen concentration, since glycogen is the main stored food reserve of the oyster. Glycogen concentrations were made using the method of Carroll et al. (1956). Individual oysters were weighed, homogenized, and the glycogen extracted three times with 5% trichloroacetic acid. Duplicate one ml samples of this acid filtrate were treated with 95% ethanol, precipitating the glycogen. After centrifugation and discarding of the supernatant, the glycogen was dissolved in distilled water. Anthrone was treated with 95% ethanol, precipitating the glycogen. After centrifugation and discarding of the supernatant, the glycogen was dissolved in distilled water. Anthrone reagent was added to these duplicate samples as well as to a reagent blank of distilled water alone and a standard glucose solution containing one-tenth milligram of glucose. After heating, these solutions were transferred to colorimeter tubes and read in a Bausch and Lomb Spectronic 20 spectrophotometer at 620  $\mu$ , after adjusting it with the reagent blank. The glycogen concentration was then determined using the equation:

$$\frac{DU}{DS} \times 0.1 \times \frac{\text{Vol. Extract}}{\text{Gm. of Tissue}} \times 100 \times 0.9 = \frac{\text{Mg. of Glycogen}}{100 \text{ Gm. of Tissue}}$$

(Carroll et al., 1956)

Where DU is the optical density of the unknown, DS is the optical density of the standard; 0.1 is the number of milligrams of glucose in two milliliters of standard solution; 0.9 is the factor for converting glucose to glycogen.

During March through June 1970, fifteen oysters from each station were brought into the laboratory for purposes of induced spawning. Following the methods of Loosanoff and Davis (1963), the oysters were subjected to systematic treatment with thermal and chemical stimuli. These spawning attempts provided direct evidence of gonadal development.

Significant differences at the .05 level between means of shell height, wet weight, dry weight, percent water, and glycogen concentration were determined using the least significant difference (LSD). Where the LSD ranges are mutually exclusive, significant differences exist at the .05 level.

## RESULTS

## Temperature

Figure 3 represents the mean weekly temperature for the power plant intake and for the mouth of Island Creek for the period June 1, 1970 through June 6, 1971. Table 1 lists mean values. Temperatures recorded for the intake station were available for fifty of the fifty-three weeks of the study. The temperature increased until late August 1970 when the highest mean temperature was recorded at the intake ( $29.0^{\circ}\text{C}$ ). A general decline in temperature occurred until early January 1971 when the lowest mean temperature ( $-1.4^{\circ}\text{C}$ ) was recorded at the intake. Following this, increases in mean temperature were the trend through June 1971.

Temperature data for the Island Creek station were available for only 34 of the 53 weeks of the study. Temperature trends at the Island Creek station generally paralleled those at the intake, but temperatures were higher. A mean temperature of  $32.4^{\circ}\text{C}$  was recorded at Island Creek and it occurred during mid-August 1970. The minimum ( $7.0^{\circ}\text{C}$ ) occurred during mid-January 1971. The maximum difference between these two stations ( $\Delta t$ ) occurred during

FIGURE 3  
WATER TEMPERATURE

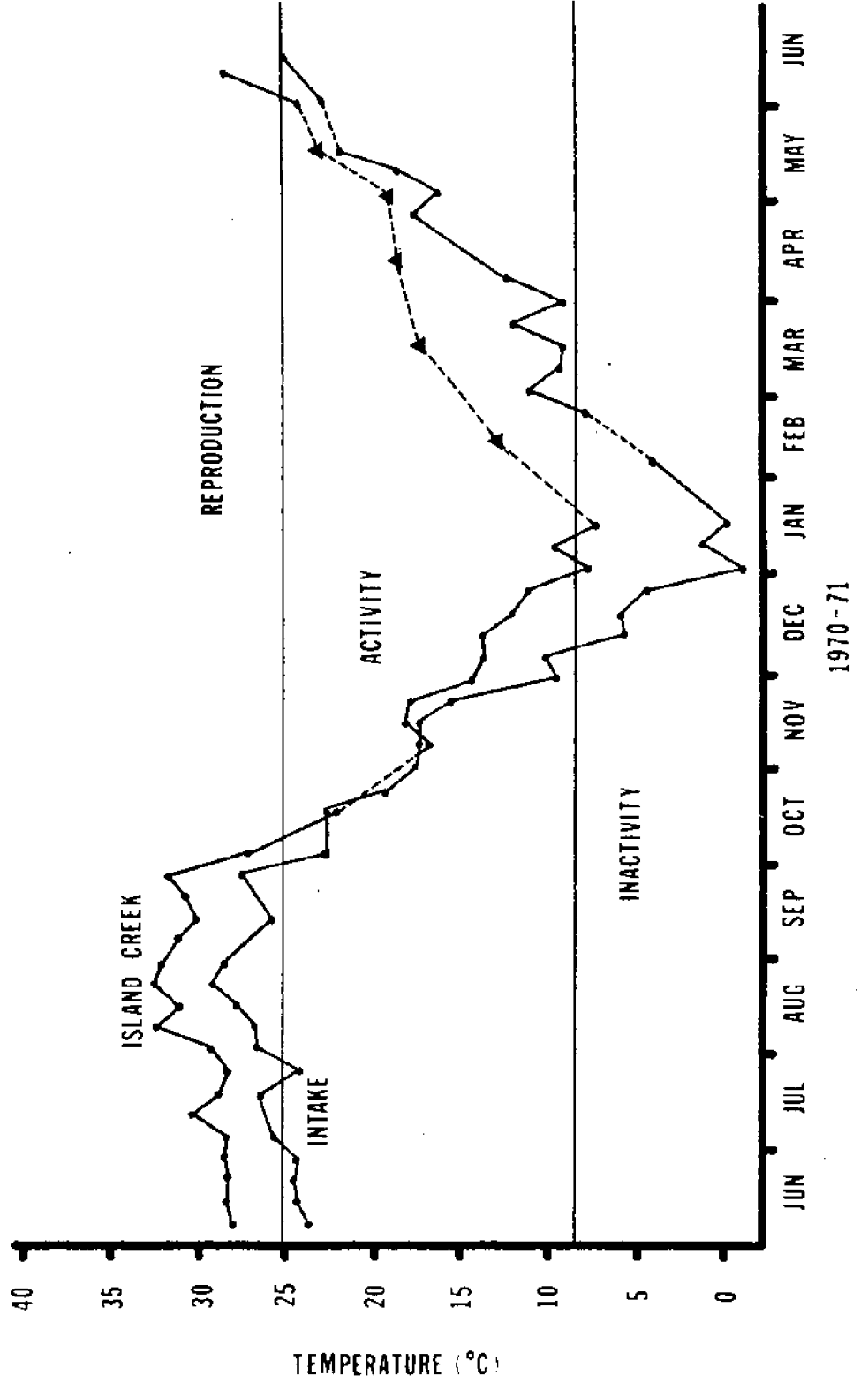




TABLE 1

Weekly Mean Temperature Readings (°C) for the  
Power Plant Intake and Island Creek  
(Delmarva Power and Light Co.)

Week No.	Intake Island Creek		Week No.	Intake Island Creek	
	1	2		1	2
1	23.5	28.0	2	24.2	28.2
3	24.2	28.2	4	24.6	28.3
5	25.6	28.3	6	26.0	30.3
7	26.3	28.7	8	24.0	28.2
9	26.6	29.1	10	26.7	32.4
11	27.6	30.9	12	29.0	32.4
13	28.3	32.0	14	27.0	31.2
15	25.7	29.9	16	26.5	30.4
17	27.7	31.6	18	22.6	26.8
19	22.4	24.4	20	22.3	21.7
21	19.2	--	22	17.5	--
23	17.0	16.8	24	17.3	17.9
25	15.4	17.6	26	9.4	14.2
27	9.9	13.5	28	5.4	13.6
29	5.7	11.8	30	4.3	10.9
31	-1.4	7.3	32	1.1	9.5
33	-0.4	7.0	34	1.1	--
35	2.4	--	36	4.0	--
37	--	--	38	7.6	--
39	10.9	--	40	9.2	--
41	8.8	--	42	11.8	--
43	8.7	--	44	11.8	--
45	13.6	--	46	15.8	--
47	17.6	--	48	16.1	--
49	18.5	--	50	21.9	--
51	--	--	52	22.8	23.9
53	24.9	27.1			

early January 1971 when the difference in mean weekly temperatures was 8.7°C.

Hydrographic data taken on monthly field trips are presented in Table 2.

#### I. Group One Oysters

Group One oysters placed in the field in June 1970 were sampled only four months (August, September, October, November, 1970) and for this reason have limited value. However, these data show some initial trends. Tables containing Group One data are presented in Appendix A. Some reference is made to them throughout this account; but in general, discussion will be limited to Group Two oysters which were sampled for ten months and, therefore, are more clear-cut in showing trends than Group One oysters.

#### II. Group Two Oysters

##### Mortality

Monthly oyster mortalities are presented in Figure 4. Mortality is expressed as a percentage of the oysters remaining per station each month. The numerical values of these mortalities are presented in Table 3. Oyster mortalities were highest at the intake in August, declining steadily in September through December. The lowest rate of oyster mortality at the intake occurred in December and February. Compared with the other stations, the intake

TABLE 2

## Hydrographic Data--Temperature (°C)

Station	1	2	3
June	28.0	35.0	24.5
July	29.8	35.5	25.3
August	30.1	39.5	27.0
September	15.0	19.5	13.0
October	15.5	19.0	14.0
November	9.0	14.0	11.5
December	2.0	8.5	2.0
January	2.5	13.0	2.5
February	11.5	17.0	11.0
March	19.5	18.5	14.0
April	15.5	22.0	14.9
May	29.0	36.3	29.5
Mean Salinity (PPT)	16.1 (12.5-21.0)	18.5 (13.5-22.5)	25.5 (18.5-33.0)
Mean Dissolved Oxygen (PPM)	11.3 (8.6-16.0)	8.4 (5.1-12.0)	10.4 (9.8-11.0)

FIGURE 4  
MORTALITY

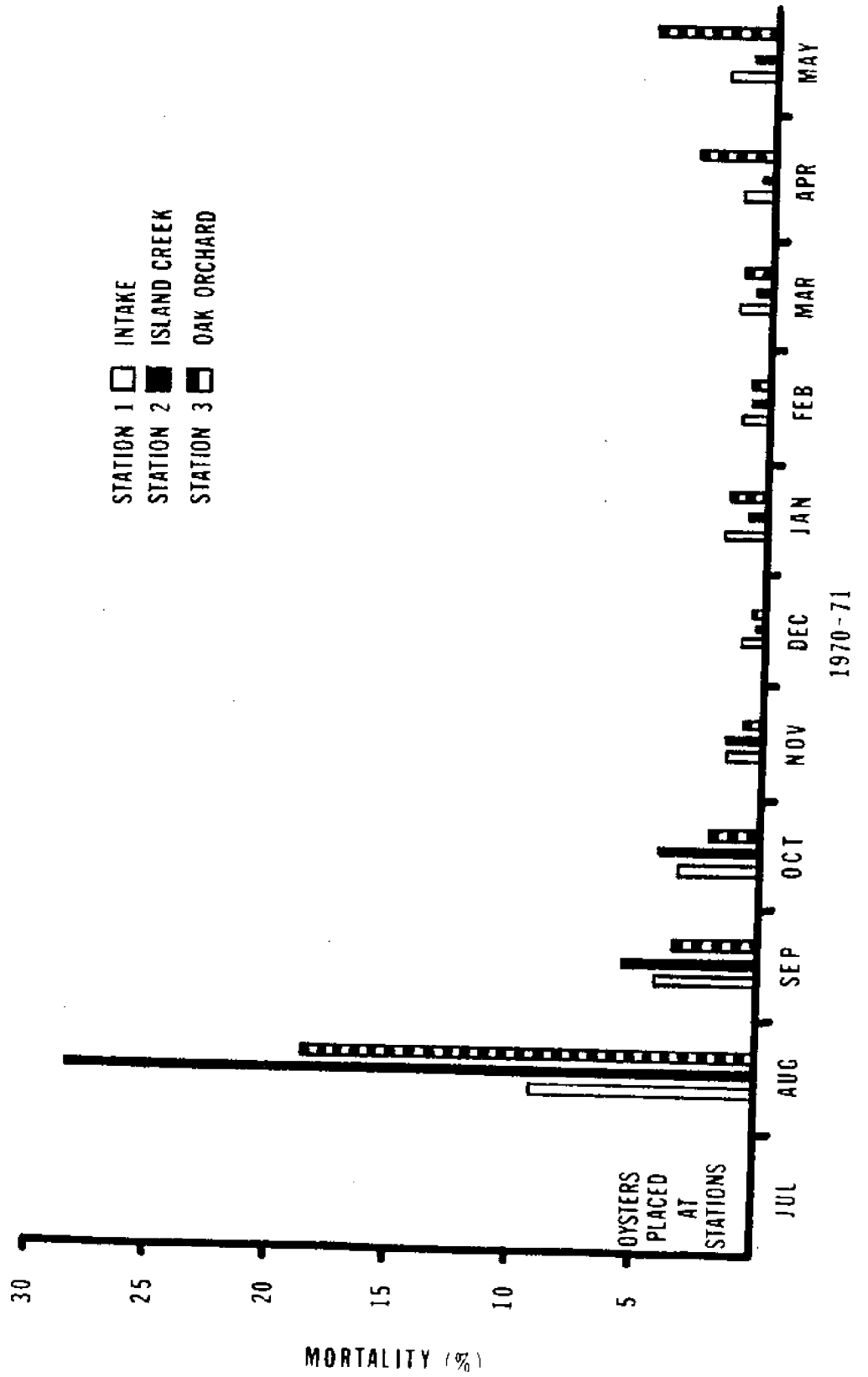


TABLE 3

Group Two--Mean Percent Mortality (%)

Station	Intake	Island Creek	Oak Orchard
	1	2	3
August	8.7	28.4	18.5
September	4.2	5.4	3.2
October	2.8	3.5	1.7
November	1.2	1.3	0.5
December	0.7	0.2	0.3
January	1.6	0.5	1.3
February	0.8	0.4	0.4
March	1.1	0.4	0.9
April	1.0	0.3	3.1
May	2.0	0.8	5.0

station showed the lowest mortality in August and September and the highest mortalities during the winter, December through March.

Mean oyster mortality at Island Creek was also very high in August, the highest observed at any station. Compared with the other stations, the Island Creek station showed the highest mortalities during August through November and thereafter showed lower mortalities than the control stations.

The mean mortality of oysters at Oak Orchard was also highest in August. In comparison with the other stations, Oak Orchard oysters showed lowest mortalities in October and November, highest mortalities in April and May.

T-tests comparing mean percent mortalities among stations show that differences were significant at the .05 level during three months. In August, the Island Creek and Oak Orchard stations had higher mortality rates than did the intake station. In April and May the Oak Orchard station showed mortalities which were significantly higher than the other stations. During the period November through March, there were no significant differences in mortality between the stations.

## Shell Height

An oyster shell grows in three dimensions (height, length, and width) as well as increasing in valve thickness. Shell height is nearly always the largest dimension of an individual oyster and is always the largest mean shell dimension of a relatively large sample of oysters. In shell growth, therefore, the net change in shell height will be larger than the changes in oyster shell dimensions. If there are any changes in shell growth due to differences in station or season, they would be more likely to show up in shell height because changes in this variable are more easily detectable and most easily measured. Therefore, the discussion of shell measurements will be limited to shell height. Appendix B contains the mean monthly values for shell length, width, and thickness of the left and right valves.

Mean monthly shell heights with standard deviations are shown in Figure 5. The numerical values are presented in Table 4. Oysters sampled from the intake increased gradually in mean shell height through November. Not until the May collection did the mean shell height exceed the November level. The May mean shell height was the largest recorded for the intake station.

The Island Creek mean oyster shell height increased through December and remained at nearly the same level

FIGURE 5

SHELL HEIGHT

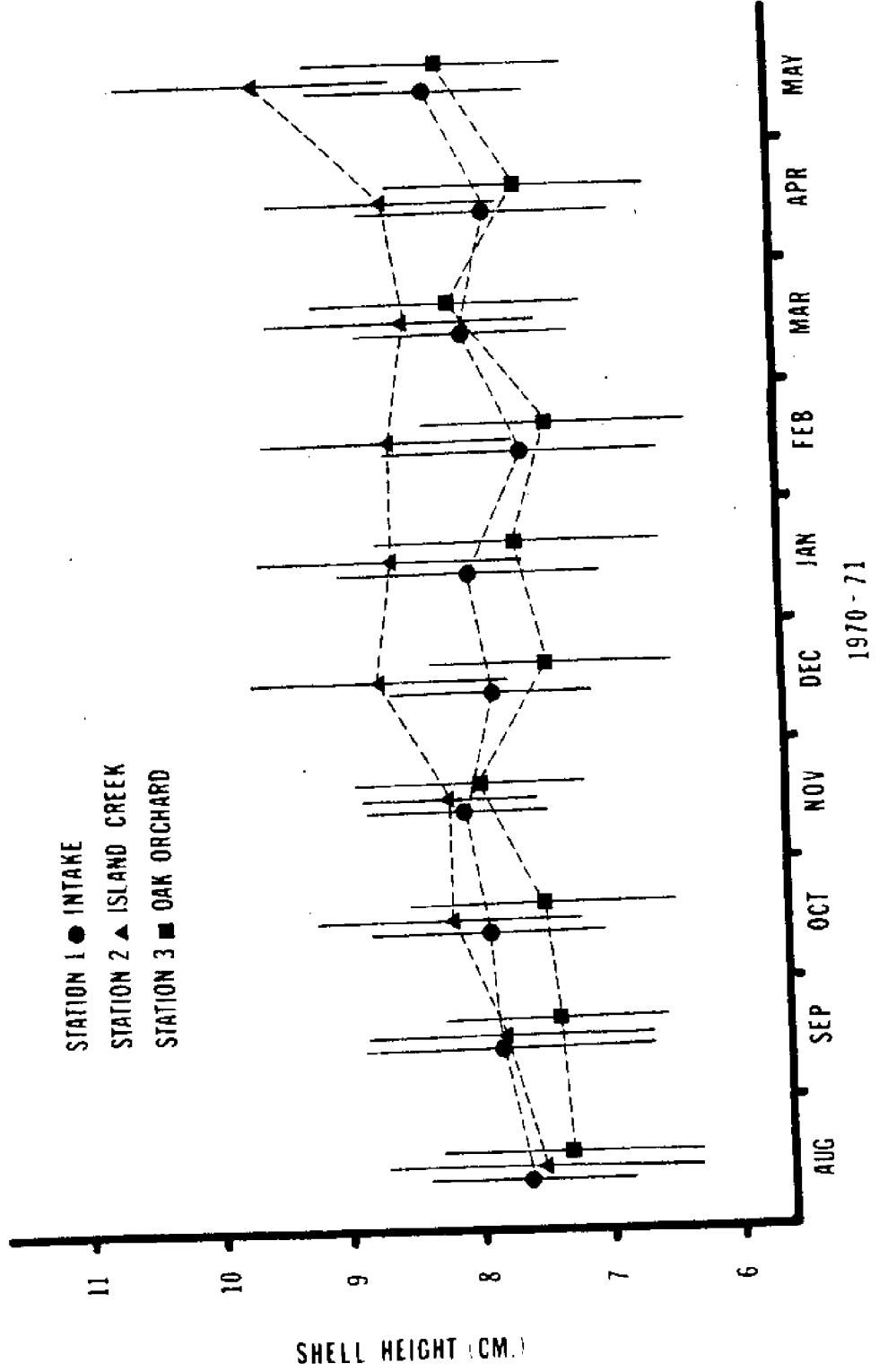




TABLE 4

Group Two--Mean Shell Height (cm)  
Oysters subsampled from those collected from Murderkill River  
June 1970 - 6.6

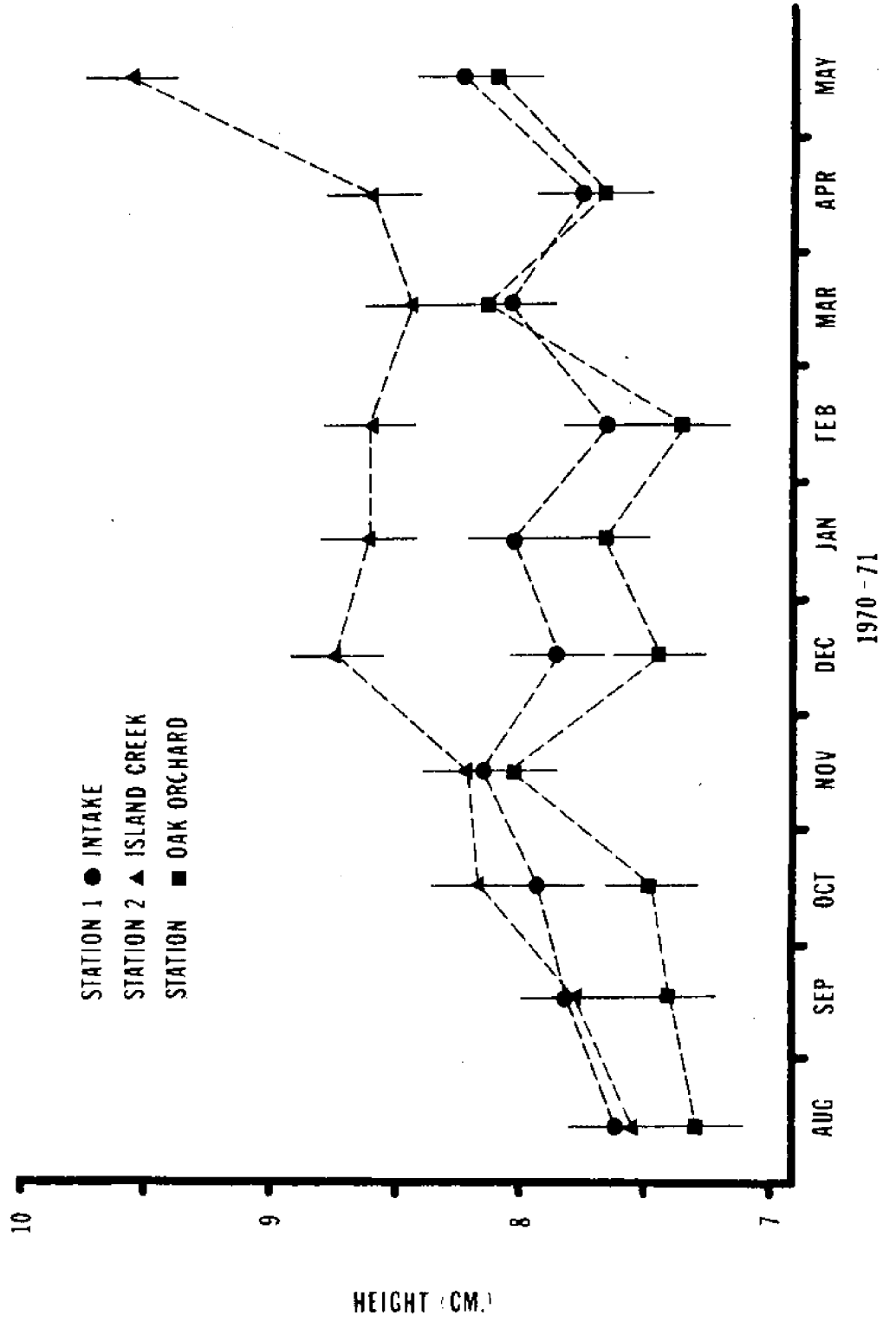
	Intake	Island Creek	Oak Orchard
Station	1	2	3
August	7.6	7.5	7.3
September	7.8	7.8	7.4
October	7.9	8.2	7.5
November	8.1	8.2	8.0
December	7.9	8.7	7.4
January	8.0	8.6	7.7
February	7.6	8.6	7.3
March	8.0	8.5	8.1
April	7.8	8.6	7.6
May	8.3	9.6	8.1

throughout January and February. The May value is the highest recorded for the Island Creek station.

Oak Orchard oysters showed fluctuations in mean shell height which were similar to those of the intake station. There was an increase which continued through November. Only in March and May was the November value exceeded. The maximum mean shell height measured at the Oak Orchard station occurred in March.

In comparing the three stations, with very few exceptions, the oysters at the Island Creek station showed the highest mean shell height and those at Oak Orchard showed the lowest mean shell height, while the shell heights of oysters at the intake were intermediate. Figure 6 shows the mean monthly shell heights of oysters sampled from the three stations. Ranges around each mean indicate the least significant difference. This is the least difference necessary to show a significant difference between means of the various stations at the .05 level. In December through February and April through May, oysters from Island Creek were significantly larger than those from control stations. Oyster shell heights varied among stations and these differences changed with time. Therefore, both station and season must be considered in evaluating changes and differences in shell height. The greatest differences in mean shell height between the ambient water stations and the effluent station occurred in December and May.

FIGURE 6  
SHELL HEIGHT - LSD



### Wet Meat Weight

Figure 7 shows the mean monthly wet weights with standard deviations. Table 5 presents the mean wet weight values. The mean wet weight of oysters sampled from the intake station increased steadily from August through November. A further increase in January produced the maximum mean wet weight measured at this station. After this peak, the general trend shows a slow decline in mean wet weight to the May level.

Oysters taken from Island Creek in August showed the lowest mean wet weight recorded for oysters from Island Creek throughout the study. A dramatic increase in mean wet weight occurred from August to a maximum in January.

Oysters from Oak Orchard increased in mean wet weight in August and September, declined in October, and increased dramatically in November, the maximum mean wet weight recorded for this station.

During the period December through May, Island Creek oysters were consistently highest in mean wet weight, Oak Orchard oysters were lowest, and intake oysters assumed an intermediate position. This shows a reversal of the early trend as Island Creek oysters were lowest during August, September, and November.

Figure 8 shows the mean wet weights of oysters sampled from each station. In October and March, oysters were significantly lighter than those from the intake or

FIGURE 7

WET WEIGHT - LSD

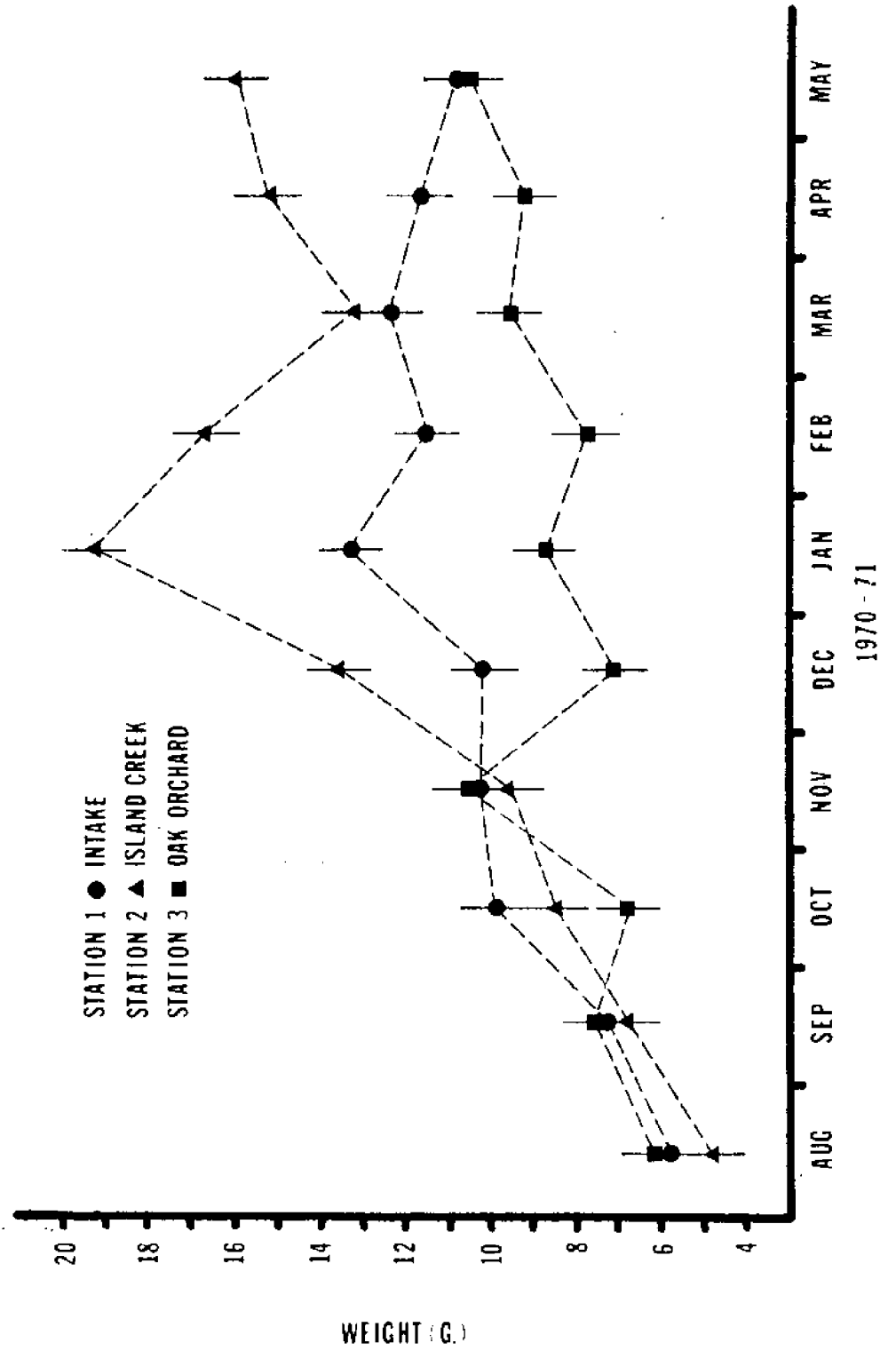


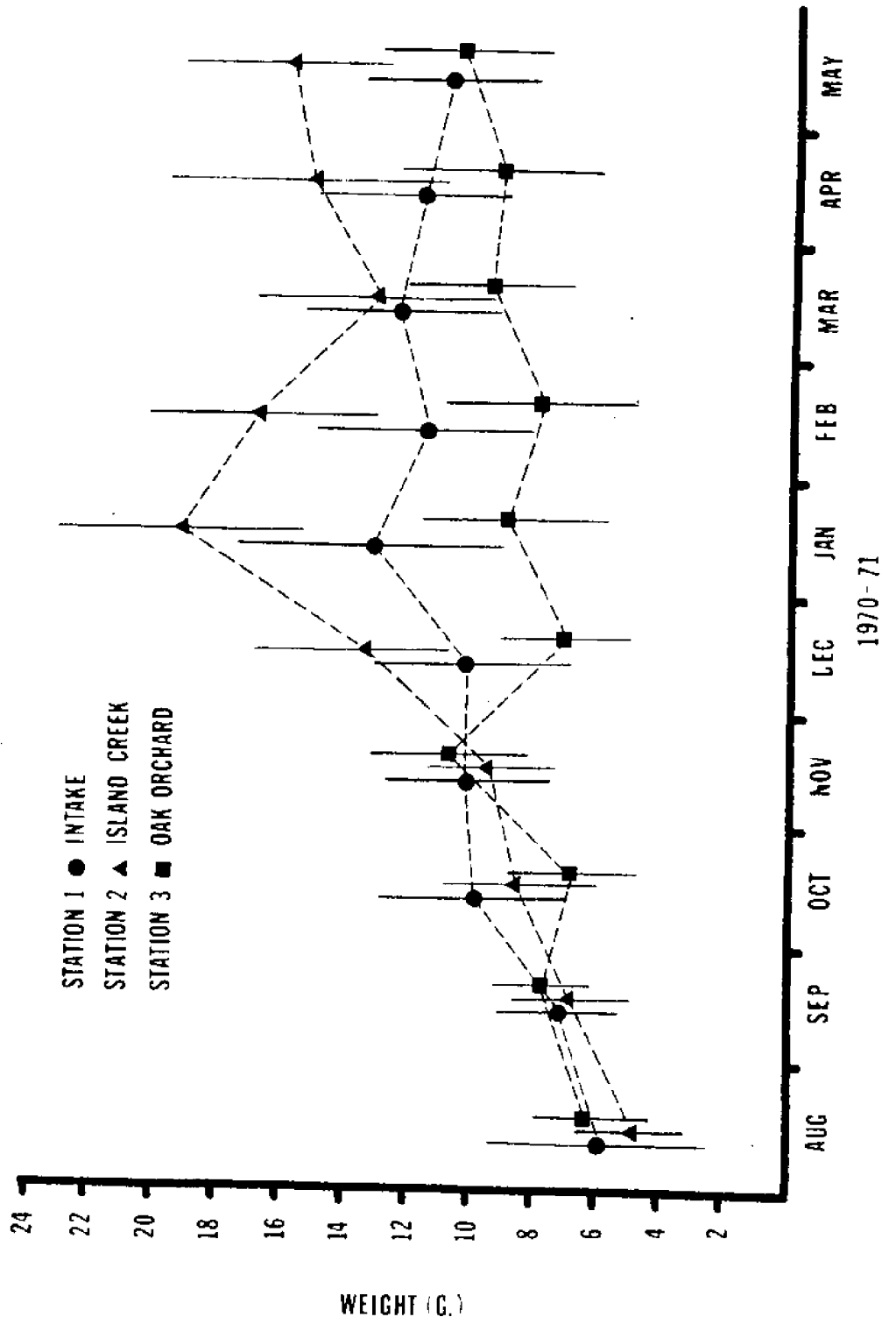
TABLE 5

Group Two--Mean Wet Weight (g)  
 Oysters subsampled from those taken from the Murderkill River  
 June 1970 - 5.3

	Intake	Island Creek	Oak Orchard
Station	1	2	3
August	5.8	4.9	6.1
September	7.2	6.8	7.7
October	9.9	8.5	6.9
November	10.1	9.5	10.7
December	10.1	13.7	7.0
January	13.4	19.3	8.8
February	11.6	16.7	7.9
March	12.4	13.1	9.6
April	11.8	15.3	9.3
May	10.8	15.9	10.5

FIGURE 8

WET WEIGHT



Island Creek. In May, Island Creek oysters were significantly higher in wet weight than those from control stations. In December through February and in April, all wet weight means were significantly different from one another; and in each case, oysters held in the effluent were heaviest while those from Oak Orchard were lightest. Both station and season had an effect on mean wet weights and therefore it is necessary to consider both in interpreting changes and differences in mean wet weight.

#### Dry Meat Weight

Values for the mean dry weights of oysters are presented in Table 6. Figure 9 shows mean dry weights. Intake oysters increased gradually in dry weight into October. The April level was the highest measured at the intake station.

Oysters sampled from Island Creek increased substantially in mean dry weight from September to February. The mean dry weight of oysters at Island Creek reached the maximum value recorded in February.

Oysters sampled from the Oak Orchard station increased from August to November. The November value was the highest mean dry weight measured at the Oak Orchard station. Dry weight showed an upward trend again in the March through May collections.

Figure 10 shows these values plotted with LSD ranges.



TABLE 6

Group Two--Mean Dry Weight (g)  
 Oysters subsampled from those taken from the Murderkill River  
 June 1970 - 1.0

	Intake	Island Creek	Oak Orchard
Station	1	2	3
August	1.3	1.0	1.0
September	1.5	1.5	1.5
October	2.6	2.1	1.7
November	2.4	2.6	2.7
December	2.1	3.1	1.3
January	2.3	3.7	1.6
February	2.5	3.8	1.4
March	2.5	3.3	1.9
April	3.1	3.2	2.1
May	2.7	3.3	2.5

FIGURE 9

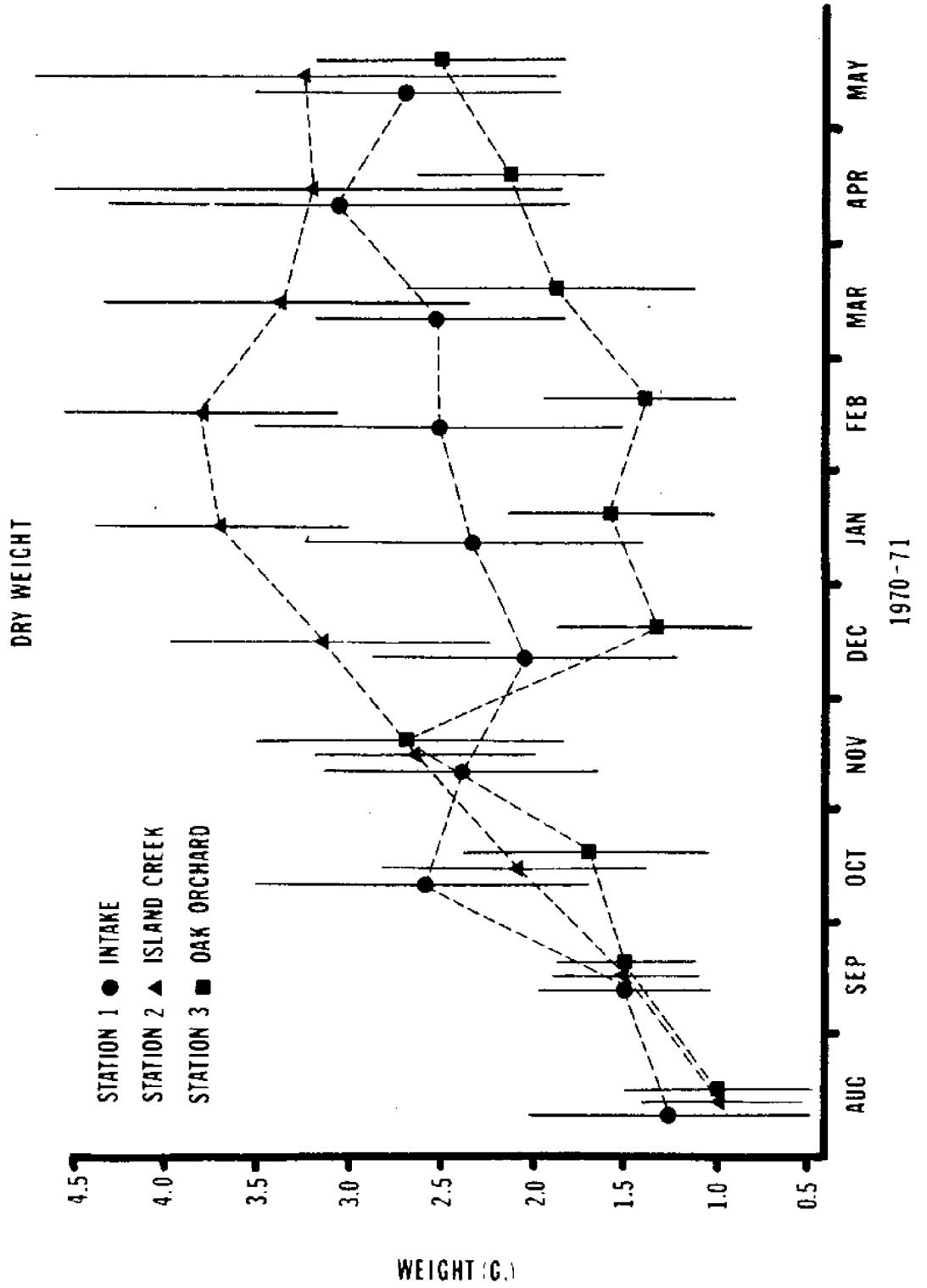
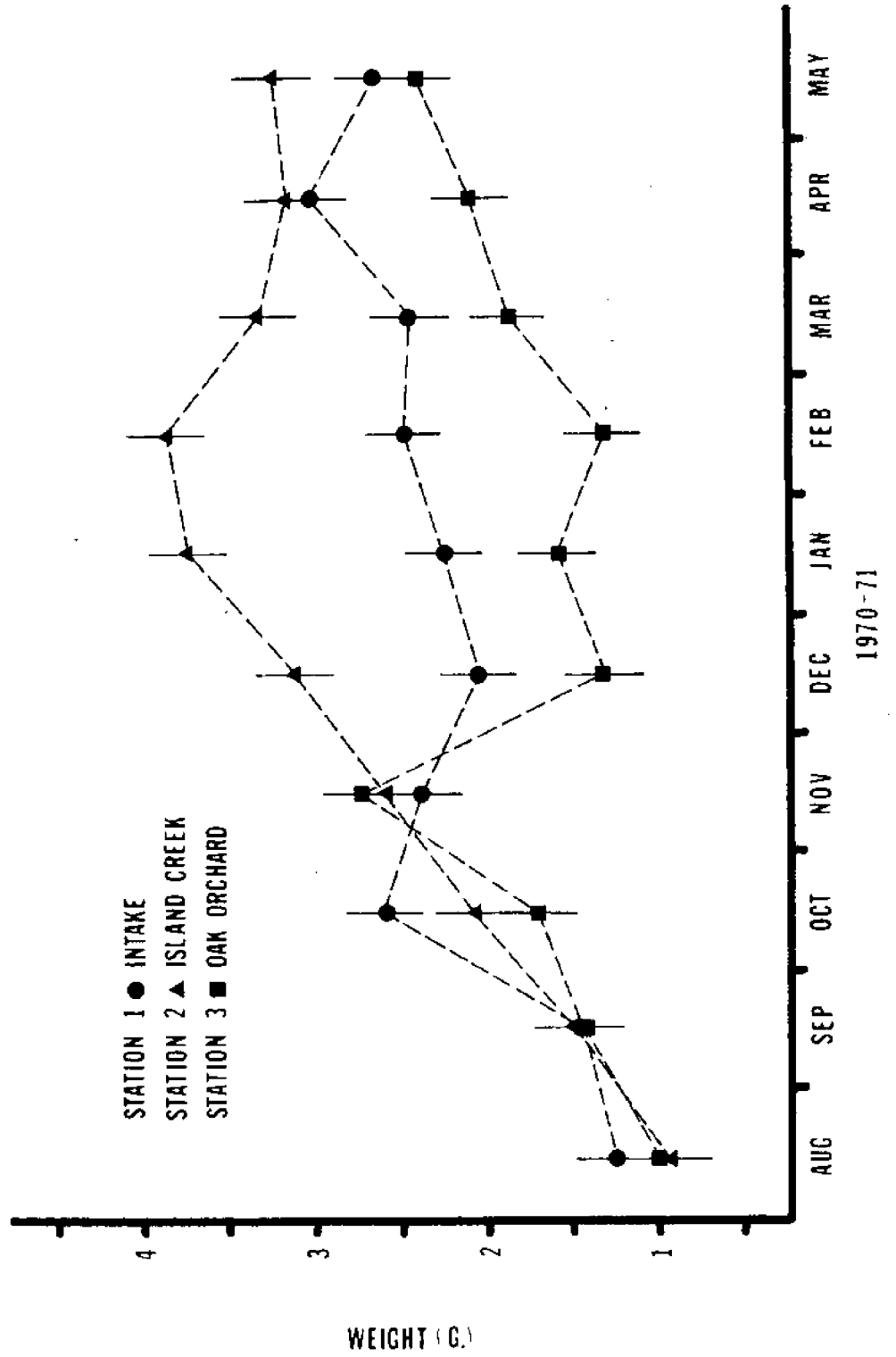


FIGURE 10  
 DRY WEIGHT - LSD



In October, oysters from Station One were significantly higher in dry weight than oysters from Stations Two and Three. In April, oysters from Station Three were significantly lower in dry weight than those from Stations One and Two. In May, oysters from the effluent were significantly higher in dry weight than those from control stations. In December through March, differences between all stations were significant; and in each case, Island Creek oysters were heaviest and Oak Orchard oysters showed the lowest wet weights. Typically, dry weights vary between stations. This variation is not a constant one, but it changes with the season. Since station and season combine to influence oyster dry weight, both must be considered in interpreting differences and changes in mean dry weight.

In comparing the three stations, distinct trends did not emerge until December. From December to May, Island Creek oysters were highest and Oak Orchard oysters lowest in mean dry weight. Intake oysters assumed an intermediate position.

#### Percent Water

Figure 11 shows the mean monthly percent water concentrations. Values for monthly means are presented in Table 7. The mean percent water value of oysters at the intake declined through October and were the lowest recorded

FIGURE 11

PERCENT WATER

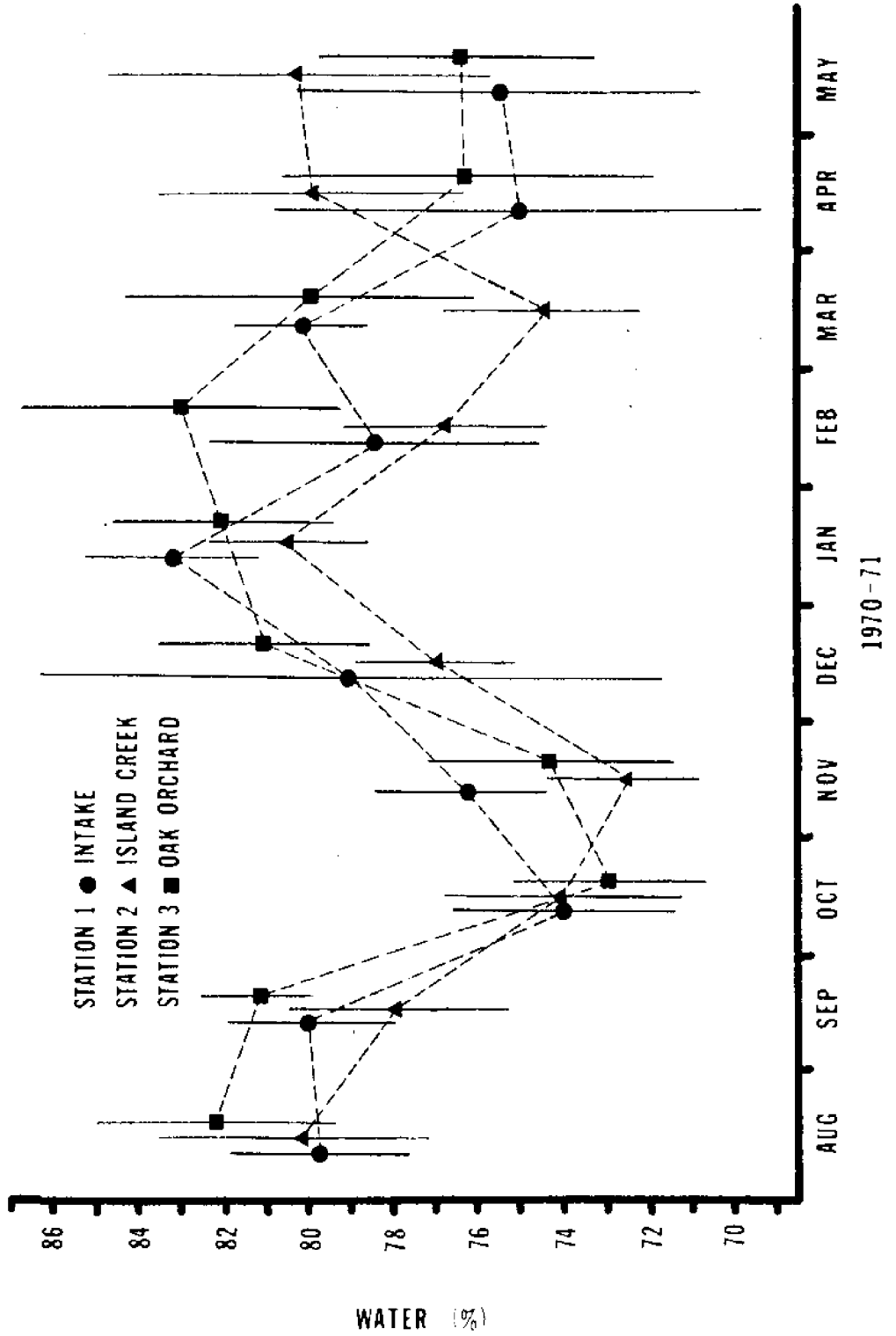


TABLE 7

Group Two--Mean Percent Water  
 Oysters subsampled from those taken from the Murderkill River  
 June 1970 - 81.1

	Intake	Island Creek	Oak Orchard
Station	1	2	3
August	79.7	80.4	82.2
September	79.9	78.0	81.2
October	74.1	74.3	73.0
November	76.4	72.6	74.3
December	78.9	77.1	81.2
January	83.2	80.5	82.3
February	78.4	76.8	83.1
March	80.2	74.5	80.2
April	75.1	80.0	76.4
May	75.6	80.3	76.5

at this station. During November through January, there was a dramatic increase to the maximum level measured at this station.

Island Creek oysters declined in mean percent water through November which was the lowest level recorded at Island Creek. Percent water values were highest in Island Creek in August, January, and May.

Oak Orchard oysters increased in mean percent water in August, then decreased to October, the lowest level measured at this station. The percent water level reached its maximum in February.

Figure 12 shows the monthly mean percent water concentrations of oysters at each station with LSD values. Oysters from Island Creek were significantly lower than those from other stations in percent water in September and March and significantly higher in April and May. Oysters from Station One showed higher percent water in November, while oysters from Station Three were higher in December and February. The most significant differences between the ambient water stations and the effluent station occurred in March.

#### Glycogen Concentration

Figure 13 shows mean glycogen concentrations. Mean values are presented in Table 8.

The mean glycogen values of oysters from the intake

FIGURE 12  
PERCENT WATER - LSD

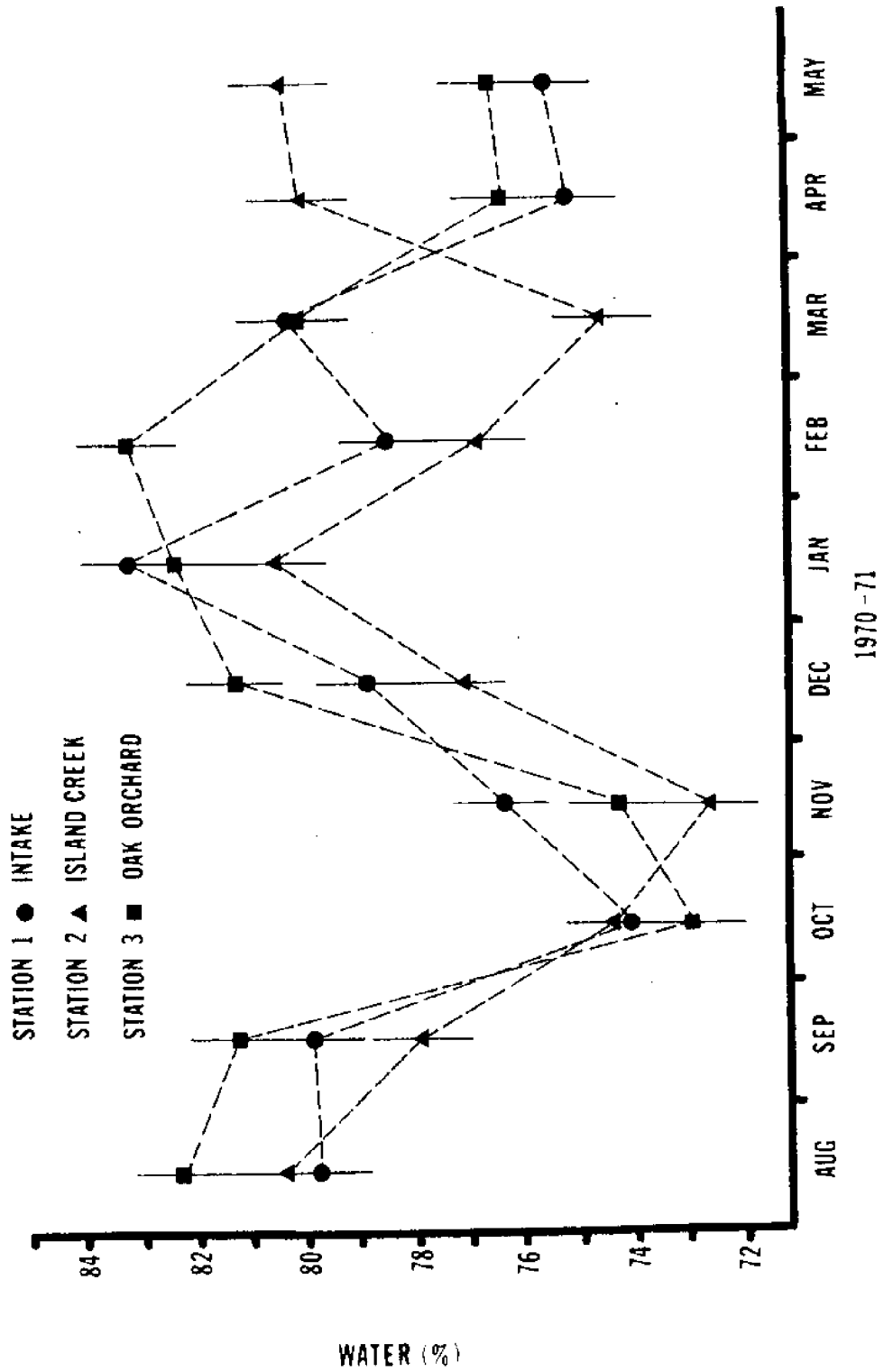




FIGURE 13

GLYCOGEN CONCENTRATION

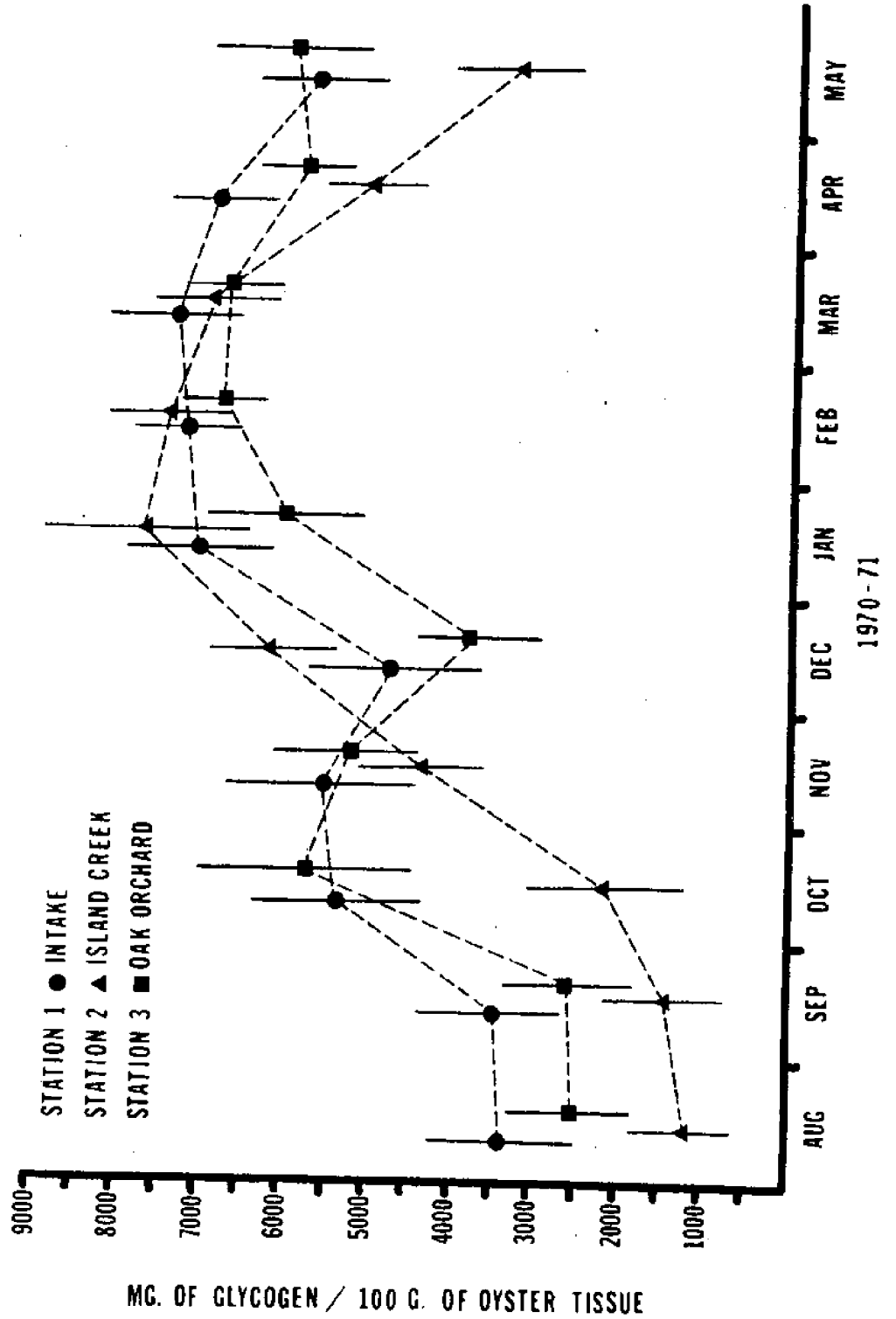


TABLE 8

Group Two--Mean Glycogen Concentrations  
 (mg of glycogen/100 g of oyster tissue)  
 Oysters subsampled from those taken from the Murderkill River  
 June 1970 - 1747.6

	Intake	Island Creek	Oak Orchard
Station	1	2	3
August	3355.4	1221.7	2506.7
September	3446.7	1436.3	2537.9
October	5324.7	2113.9	5699.3
November	5499.4	4323.4	5200.1
December	4667.1	6099.1	3666.6
January	7010.1	7668.4	6000.1
February	7196.6	7405.1	6751.1
March	7323.0	6885.0	6665.0
April	6810.3	5000.2	5850.0
May	5670.5	3299.4	6000.7

increased steadily through the November collection with another increasing trend in January through March. Glycogen levels then began to decline in April and May. Concentration at the intake was lowest in August and highest in March.

Oysters sampled from Island Creek in August showed an initial decrease in mean glycogen levels. Levels rose through January. The lowest concentration was in August, the highest occurred in January.

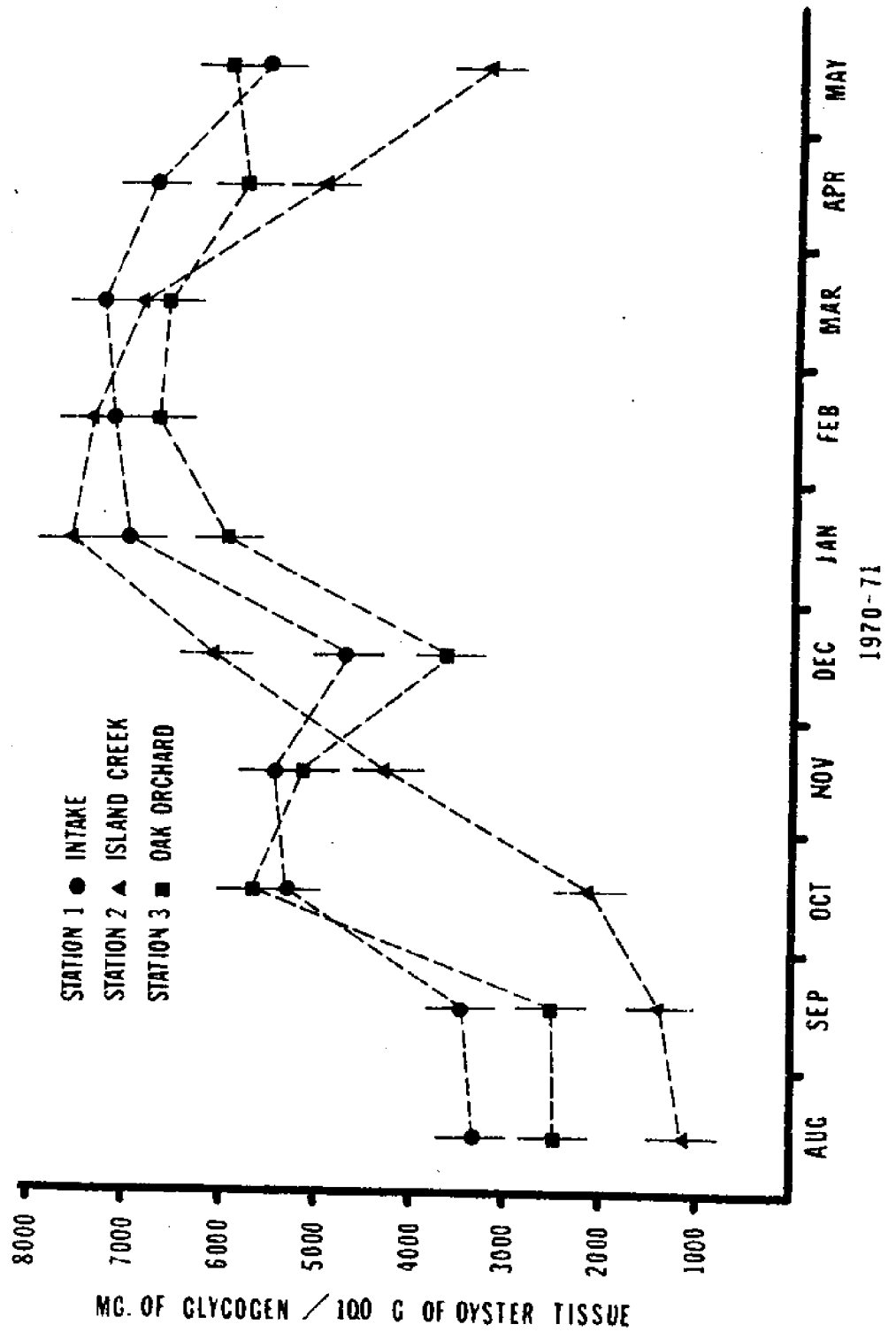
Oysters sampled from Oak Orchard showed an increase in mean glycogen level through October. The lowest reading occurred in August and the highest in February.

Figure 14 shows mean monthly glycogen concentrations for the three stations with the least significant differences. In October and November, oysters from Island Creek were significantly lower than control stations in glycogen reserves. In January, oysters from Station Three were significantly lower in glycogen than those from Stations One and Two. In May, oysters from Island Creek were significantly lower in stored glycogen than control stations. Glycogen concentrations varied between stations and with the season; this interaction must be considered in evaluating glycogen concentrations.

In comparing the effluent station with ambient water stations, Island Creek oysters showed highest glycogen

FIGURE 14

GLYCOGEN CONCENTRATION - LSD



concentrations from December through February. During the warmer months, Island Creek oysters are generally lowest in glycogen.

The greatest differences in glycogen concentration between stations occurred in October, May, and August. In each case, the Island Creek levels were lower than ambient water stations.

#### Spawning

During April, May, and June of 1971, fifteen oysters from each station were returned to the laboratory periodically for artificial spawning experiments. In April, attempts were made to spawn oysters from each station during the period April 15-17, 1971. Each group of oysters was exposed to two full days of elevated temperatures (26-30°C). On the second day, sperm suspensions were added to each group. Spawning attempts were unsuccessful. Gonads were generally not well developed and sperm were inactive.

Similar spawning attempts were made May 6-8, 1971. Intake oysters appeared to be in good condition with firm developing gonads, but sperm were only slightly active and eggs were immature. No intake oysters spawned in response to elevated temperatures or sperm suspensions. Oak Orchard oysters were generally in good condition with fair gonad development, but sperm were inactive and eggs were immature. No Oak Orchard oysters were induced to

spawn. Island Creek oysters were in excellent condition with good gonad development and active sperm. On the second day, two hours after introduction of a sperm suspension, one male and one female of a total of 15 oysters spawned. The eggs were fertilized and developed normally.

When a third and final spawning attempt was made, June 7-9, 1971, no oysters from any of the stations were induced to spawn. Island Creek oysters were in fair to good condition and many had very active sperm and well formed eggs. Intake oysters were in generally good condition. Eggs were immature and sperm were active in only some individuals. Oak Orchard oysters were in fair to good condition; sperm were inactive and eggs were immature.

## DISCUSSION

Temperature is generally considered to be the major environmental factor influencing poikilotherms (Gunter, 1957; Kinne, 1963). Mihursky et al. (1970) list three types of effects of temperature on aquatic organisms: (1) lethal effects resulting in mortalities, (2) directive effects influencing activities such as spawning, and (3) controlling effects influencing the rate of metabolism. Upper and lower temperature tolerances vary from one species to another and may depend upon the age and condition of an organism, its geological history as a species, or the environmental regime to which it has been acclimatized. Thermal limits tend to increase with age. Crassostrea virginica larvae develop within rather narrow temperature limits (Loosanoff and Davis, 1963), while adults experience ambient water temperatures of 1-36°C within their geographical range (Galtsoff, 1964). The influence of geological history on cell thermostability can be seen in the work of Zhirmunskii (1966) on Crassostrea gigas. Organisms from northern Siberia were found to have a lethal temperature of 47°C despite the cold climate of this area. This was explained on the basis of the subtropical climate of

this area during the Miocene epoch. The environmental conditions to which an individual or a species has become accustomed are also important in determining thermal tolerance limits. Adaptation to a certain temperature regime may occur on several levels. A genetic change brought about by natural selection within a species may lead to the establishment of physiological races which are separated geographically. Stauber (1950) proposed the existence of physiological races of oysters based on spawning temperatures of different populations along the east coast. Loosanoff and Nomejko (1951) concurred in showing that southern oysters failed to spawn at ambient summer temperatures in Connecticut, nor could they survive the severe winter conditions in this latitude. Within species or physiological races, thermal tolerances may be affected by acclimation to various temperature regimes. Many poikilotherms exhibit a non-genetic compensation in their metabolism or activity which gives them a degree of independence of temperature (Bullock, 1955). This compensation is acclimation. Although the ability to acclimate is far from universal, many molluscs show a varying degree of homeostasis of rate functions and shifts in tolerance levels in response to seasonal or latitudinal temperatures (Segal, 1961). The mechanism of thermal acclimation has not been elucidated. Although it may proceed simultaneously, at varying speeds, and on



several levels (Somero, 1969), involving hormonal and nervous factors (Lagerspetz and Tirri, 1969), the retention of acclimation in various isolated cells and tissues points to the cell as the site of acclimation (Segal, 1961; Vernberg et al., 1963; McWhinnie, 1967).

Temperature also affects the geographical distribution of a species (Hutchins, 1947). Oysters are limited as a community, dominant in their distribution toward the equator because of summer temperatures which cause more and more stress as one goes south (Collier, 1951). Oysters are limited in their poleward distribution by the minimum summer temperatures in these areas necessary for gonad development and spawning to occur.

Acclimation, physiological race, geological history, condition, and age tend to affect thermal tolerances of an individual only within certain limits for a given species. As the temperatures rise past the optimum level, metabolism becomes inefficient and oxygen consumption often declines, showing that the organism is under stress. When the organism fails to respond normally to stimuli, it is said to be experiencing heat coma or ecological death. This state continues as the temperature rises to the critical lethal temperature known as the thermal death point or physiological death point. Death at this temperature may be due to insufficient oxygen, decreased viscosity of protoplasm, osmoregulatory difficulties due to increased cell membrane

permeability, toxins from damaged cells, or to the inactivation of enzymes exceeding synthesis (Kinne, 1963). Yokota (1953) found that summer mortalities in Ostrea (Crassostrea) gigas were due to accumulation of harmful autolysis products. Drost-Hansen (1969) stated that thermal death may be due to changes in the structure of vicinal water within the cell.

During summer months, when ambient temperatures are normally high, the addition of waste heat to a body of water may elevate temperatures beyond the thermal death point of some organisms. Kennedy has demonstrated this with the soft clam, Mya arenaria (L.) in Chesapeake Bay (Kennedy, 1967; Kennedy, 1968; Kennedy and Mihursky, 1971; Kennedy and Mihursky, 1972). In this latitude, Mya is at the southern limit of its range and therefore lives near its upper thermal tolerance during summer months.

#### Mortality

Mackin (1961) listed mortalities due to extremes of the physical environment as Type I mortalities. The oyster, like most widely distributed, sessile, littoral, or intertidal zone organisms, is eurythermal. Galtsoff (1964) reports that intertidal Gulf Coast oysters survive temperatures of 46-49°C when exposed at low tide. Ingle et al. (1971) report survival of intertidal Gulf Coast oysters at temperatures of 49.5°C even when temperatures exceeded

44°C for three hours. Henderson (1929) gave 48.5°C as the thermal death point of Crassostrea virginica. Vernberg et al. (1963) showed that excised oyster gills lived for some time at 44°C. Other intertidal organisms show similar resistance to high temperatures. Crassostrea gigas (Thunberg) showed cell thermostability up to 47°C (Zhirmunskii, 1966). Hoshi and Hoshiyama (1963) found that isolated gills of Mytilus edulis lived at 45°C. Orr (1955) reported survival of Uca and Nassa at 46°C. On the basis of these studies, it seems probable that the critical lethal temperature of the oyster was not reached at the effluent station.

A more likely cause of the high summer mortalities is thermal stress. The highest mortalities in natural populations of oysters are usually associated with the warmest parts of the year (Collier, 1951; McHugh and Andrews, 1954). This may be due to the inability to cope with the physiological stress associated with elevated temperatures following spawning. Spawning requires energy and calls upon the oyster to mobilize stored reserves in the formation of gonad and gametes. This demand decreases stored glycogen reserves to a low level during the warmest season of the year when metabolic demands are highest.

Oysters held in the Delmarva Power and Light Company effluent during summer months would often be exposed to temperatures between 30-40°C. There is abundant literature to show that the optimum temperature range of the oyster

is below this level. Collier (1951) showed that for Gulf Coast oysters the optimum pumping rate occurred between 20-25°C. Above this temperature, pumping decreased. Loosanoff (1958) working with Long Island oysters found that the pumping rate of the oyster declined above a maximum at 31-32°C and showed severe stress above 34°C. Oysters at 32°C seemed to be unfavorably affected and the optimum level was considered to be near 25°C. This was true regardless of acclimation to the experimental temperatures. Galtsoff (1927) found that the activity of the gill cilia of C. virginica increased from 6-31°C and then declined sharply in response to further heating. Federighi (1929) found that the heart-beat of C. virginica increased with temperature to 30°C, but declined at higher temperatures. Others have found similar results with other related species. Hopkins (1935) stated that the maximum pumping rate of C. gigas occurred at 27°C; however, the optimum temperature was judged to be near 20°C. Hamwi (1968) found 24-26°C to be the optimum temperature for pumping of the hard clam, M. mercenaria. Pumping ceased entirely above 32°C. Loosanoff (1942) found a decrease in the percentage of the time that the mussel, Mytilus edulis, will pump at temperatures in excess of 25°C. Based on these studies, the oyster probably experiences heat coma at temperatures in excess of 32°C. During August, the mean temperature exceeded 32°C for more than a week. Temperatures as high as 39.5°C were measured during spot check-

ing and for several days the temperature remained above 35°C throughout a twenty-four hour period. From these data, physiological stress due to the heated effluent can be strongly inferred as a major cause of summer oyster mortalities at the Island Creek station.

Some mortalities at each station may have been due to mechanical damage from collection, culling, and holding out of water. Medcof (1946, 1959) found that holding oysters out of water and transferring them from one location to another can have a detrimental effect on the fatness and survival of oysters.

Salinity shock or the combined effects of an abrupt salinity change with high temperatures can probably be eliminated as a source of mortality. Mean summer salinities from the Murderkill River oyster bar where the oysters were collected was 21 o/oo (Aprill and Maurer, unpublished data) which is similar to salinities in Indian River Bay (Station One - 16.1 o/oo, Station Two - 18.5 o/oo, Station Three - 22.5 o/oo). Some initial mortalities may have been due to temperature shock. Fingerman and Fairbanks (1956a, 1956b, 1957) found that oysters can be killed by a short and sudden exposure to temperatures well below the thermal death point for the species. This is a short term temperature stress to which the oyster is unable to become accustomed due to prior acclimation. This factor combined with relatively poor conditions following spawning may have

contributed to high initial mortalities of oysters, especially those at Island Creek. In addition, Quick (1971) found that oysters in the early stages of gametogenesis are selectively killed by sudden exposure to temperatures near 35°C.

In considering the problem of thermal disturbance in the marine environment, factors other than temperature must be considered. Chlorine and other biocides, lower dissolved oxygen levels, depressed food concentrations, trace metals from power plant heat exchangers and altered currents and sedimentation probably combine with temperature to affect organisms or their parasite-host, disease-host, or predator-prey relationships.

Chlorine is not thought to have had any detrimental effect on oysters held in the effluent due to the distance from the outfall. Holmes (1970) states that sea water has a fairly high chlorine demand and that active chlorine rapidly disappears from the system being quickly absorbed by suspended particles, dissolved sulfides, and organic matter. In addition, Waugh (1964) determined in laboratory experiments that the larvae of the oyster, Ostrea edulis (L.) would survive at ten-minute exposure to 10 ppm of chlorine at 30°C. This concentration is higher than those commonly used in power stations. The Delmarva plant chlorinates intermittently maintaining a tailpipe residual of 0.6 ppm

(Gibbons, personal communication). It is likely that active chlorine has been removed from the water by the time it reaches the mouth of Island Creek.

The worm, Polydora, may also have contributed indirectly to oyster mortality. They were found living in oyster shells at all three stations. Polydora were most numerous in Island Creek, numerous at the intake, and present to a lesser degree at Oak Orchard. Quick (1971) stated that Polydora is most prevalent during warmer months and Loosanoff and Engle (1943) stated that Polydora is usually associated with lower salinities which partially explains its distribution at our stations. Lunz (1941) stated that the oyster may be forced to spend considerable energy secreting shell to cover the worms. This demand at a time of physiological stress might contribute to some oyster mortalities.

Fouling organisms may also have contributed to the summer mortalities. Based on biomass, the barnacle (Balanus improvisus) was the major fouling organism at the intake and at Island Creek. Setting and growth were especially extreme in the effluent (Gibbons and Brady, 1971). Increased biomass of fouling organisms in heated effluents were reported by Cory and Nauman (1969). Nauman and Cory (1969) stated that Balanus set earlier in the effluent and showed increased growth. Roessler (1971) reported increased growth rates during part of the year for barnacles in an

effluent. At Oak Orchard, the major fouling organism was serpulid worms. Although fouling organisms were removed as often as possible, rapid growth of serpulids at Oak Orchard and barnacles at Island Creek caused detritus, feces and pseudofeces to be trapped, filling the sea racks with sediment. Fouling occurred at the intake to a lesser degree. Mackin (1961) listed as Type IV oyster mortalities spatial competition from epifaunal organisms such as barnacles. In a series of studies with the Japanese pearl oyster, Miyauti (1967, 1968, 1969) found that the presence of fouling organisms reduced the activity and vitality of the oyster. The growth rate declined as did condition and oxygen consumption in heavily fouled oysters. The barnacle, B. amphitrite, was found to be the most detrimental fouling organism. In addition to mortalities due to competition with fouling organisms, Mackin (1961) listed competition with other oysters and crowding as Type VII mortalities. This factor may have combined with the effects of fouling organisms to cause some mortalities as initial densities in the trays were high.

Throughout the course of the study, the waters of Indian River Bay carried large amounts of silt, especially at the Island Creek station. Loosanoff and Tommers (1948) found that even at low concentrations (0.1 G/L) suspended silt resulted in a 57% decrease in the pumping rate of the oyster. In addition, the oyster trays, especially when



heavily fouled, tended to trap sediment and many oysters may have been buried for a period of days. When living anaerobically the oyster utilizes glycogen reserves and, when they are exhausted, death will result unless aerobic respiration can be resumed. Total utilization of reserves would be more likely to happen at higher temperatures. Dunnington (1968) found that oysters forced to live anaerobically, consuming glycogen reserves, showed mortalities which varied directly with temperature. During cold winter conditions, oysters lived up to five weeks, while during the summer death occurred within a matter of days. Read (1964) found that Crassostrea rhizophorae held anaerobically at 37°C would survive for forty-eight hours, while at 40°C survival time was decreased to thirty-six hours.

Heat may have contributed indirectly to mortalities at all three stations in yet another way in its effect on several oyster diseases of this area. Mackin (1961) listed oyster mortalities due to disease as Type II mortalities. Both Labyrinthomyxa marina, a fungus disease, and Minchinia nelsoni, (MSX), a haplosporidian parasite, are active in June-August. Minchinia nelsoni causes high mortalities in July-August (Couch and Rosenfield, 1968) and Labyrinthomyxa marina causes mortalities in July-October (Andrews, 1965). Hewatt and Andrews (1957) found that temperature above 25°C was necessary to cause high level mortalities due to L. marina. A comparison of the mortalities of Group One and Group Two

oysters shows that the timing of the mortalities of these groups was different, thereby further reducing the likelihood that an oyster epizootic was responsible for the observed mortalities. Where disease organisms or parasites are present in areas where thermal stress is a problem, a sub-optimal physiological condition may be created presenting pathogens and parasites with an opportunity to overcome their hosts (Vernberg, 1969; Mihursky et al., 1970). Therefore, a thermal effluent might be expected to disturb normal pathogen-host, parasite-host relationships by maintaining temperatures at high levels for a longer part of the year. Since the ambient temperature reached 30°C at all stations, mortalities caused by these organisms would occur at all three stations if these disease organisms were present. Since mortalities were not severe at any station, it is not likely that an oyster epizootic was responsible in this case.

Group Two oyster mortalities were highest in August, the first month they were determined. Highest mortalities would be expected in August, but normally the magnitude would be expected to be less. A comparison with Group One oyster mortalities (Appendix A-1) shows high initial mortalities in June with a secondary peak in August. This level represents more normal August mortalities as the oysters had had two months to acclimate to these high temperatures.

Mortalities were low throughout the fall months reaching the lowest level at all stations in December. The physiological stress of the high ambient and effluent temperatures had been removed. Temperatures during this period favored normal pumping rates. Dame (1972) found that although the metabolic rate declines during the fall, the assimilation rate of ingested food was higher during this period. Therefore, food reserves may be stored and the oyster may become fat. Mortalities remained low throughout the winter months at all stations. Ansell (1969) and Kennedy and Mihursky (1972) warn that elevated effluent temperatures during winter months might increase the metabolic demands of shellfish at a time when food organisms would not be available causing starvation. This does not seem to be the case in Indian River Bay as meat weights and glycogen levels were high and mortalities were very low during winter months. Ayer et al. (1970) reported reduced winter mortalities for oyster spat held in an effluent in New Hampshire where winter icing was a problem. This pattern is similar to that found in our study.

Spring mortalities (April-May) were highest at Oak Orchard due probably to extensive serpulid worm fouling, which similarly to barnacles tends to trap sediment. The mortality pattern would be expected to shift with highest mortalities occurring in Island Creek later in the summer. At the time of the May collection, temperatures in excess

of 30°C were measured in Island Creek, but the short duration of these elevated temperatures had not yet resulted in higher mortalities. Roosenburg (1968) worked with transplanted oysters in a power plant effluent. He reported higher initial mortalities for oysters placed within four hundred meters of the outfall during the warmer months (May-September). Oysters transplanted between October and April showed no detrimental effects or higher mortalities than those planted at control stations. Based on our mortality results, it seems likely that a similar pattern would be found at the Indian River stations if oysters were transplanted during colder months.

The interpretation of oyster mortality in this case is rather complex. High temperature has the direct effect of imposing metabolic stress on oysters. Although these temperatures are below the critical lethal temperature for this species, oysters may be particularly susceptible to the effects of high temperatures during early gametogenesis. In addition, temperature may have secondary effects on oysters influencing the types, abundance, and growth of fouling organisms in the effluent and possibly the predators, pathogens, and parasites present and their relationship to the oyster.

### Shell Growth

As an oyster grows, the mantle secretes new shell in four distinct layers. The layer which is laid down first at the periphery of the shell is the periostracum, a thin film of organic material. Because this layer is thin, it is very fragile and any shell growth measured is that over and above breakage. New shell growth can easily be damaged by handling and cleaning the oysters. Apparent negative shell growth can be caused by breakage or to sampling of smaller oysters due to chance or a combination of both factors. Breakage is likely to exceed growth during cold weather; reports of "negative growth" during winter months are not uncommon even when large samples are involved (Beaven, 1953).

All stations showed positive changes in shell height until December. At this time, negative shell growth was first observed due to breakage and sampling problems. Oysters from Stations One and Three showed increases in height in October and November. Oysters at Island Creek showed growth which continued into December before leveling off. Because of the elevated temperatures in Island Creek, the winter was shortened or modified and the growing season was extended at this station.

Arndt (1968) reported an extension of the growing season for raft-cultured oysters held in a power plant effluent. Also, the mussel, Mytilus edulis, was observed to

grow throughout the year near the outfall. Ayer et al. (1970) found reduced winter growth of raft-cultured spat held in an effluent in New Hampshire possibly due to the fact that oysters were held very close to the outfall (10 meters) where there was low water quality due to turbulence, sedimentation, and biocides. Ansell et al. (1964) showed that heated effluents increased both the growing season and the instantaneous growth rate of clams. The thermal effluent is similar to a southerly shift in geographic range. Roosenburg (1968) found no significant differences in shell growth between oysters in the effluent and those at control stations. He felt that there were necessarily some subtle effects, but that they were masked by intrastation variation or by large differences in salinity between his stations. Oysters from Island Creek were consistently larger in shell dimensions than oysters at the other station and they showed signs of an extended growing season. Shell growth was not slowed even during August and September, the months of greatest thermal stress. This summer shell growth at Station Two may have been in response to heavy infestations of the worm, Polydora. Loosanoff and Nomejko (1949) stated that gametogenesis and spawning did not interfere with shell growth. Stewart (personal communication) reported extremely rapid growth of oyster spat in heated effluent water near 35°C at a power plant in Florida. Also, Salo and Leet (1969) report extensive shell growth throughout

the year for Crassostrea gigas held in a thermal effluent. Boetius (1962) found an accelerated growth rate of Mytilus edulis exposed to the effects of a power plant effluent.

#### Meat Weight

Increases in oyster shell dimensions do not necessarily reflect similar increases in meat weights. Oyster growth patterns vary with age, latitude, and local conditions (Butler, 1953). Meat weights must be considered together with shell dimensions in evaluating oyster growth.

The wet weight of an oyster consists largely of water, oyster tissue, and glycogen reserves. Because of the glycogen-gonad cycle in oysters, the proportions of these components vary throughout the year.

Oysters held in the effluent were lower in wet weight in August than baseline oysters measured in June even though the former showed considerable shell growth. Similarly, Ansell (1968) showed that the hard clam, Mercenaria mercenaria, held in a thermal effluent of a power station increased in shell dimensions without a similar increase in meat weight. The decline in wet weight of oysters held at Island Creek was probably due to several factors including spawning, high pumping rates and low concentrations of phytoplankton, which resulted in utilization of solid reserves. During the summer, there was some degree of thermal stress at all stations due to high ambient temperatures. This inhibited

the rate of meat weight increase at control stations.

During the fall and early winter, wet weights of oysters increased dramatically at all stations. The metabolic rate of the oyster declined with the temperature while the assimilation and growth rates increased (Dame, 1972). Gametes and gonadal tissue were resorbed and glycogen concentrations increased rapidly. Since meat weights of oysters from Island Creek were consistently greater from December through May, it is probable that this was due to greater growth of oyster tissue and not just higher winter glycogen concentrations. The spring decline in meat weights of oysters from the effluent station is due to loss of stored glycogen. This was caused by an early temperature rise in Island Creek resulting in increased metabolic demands. Moreover, the concentration of phytoplankton may not have increased as pumping rates increased. Ruddy et al. (unpublished data) held oyster spat in a heated effluent area in Connecticut. They found that wet meat weights of oysters held in the effluent continued to increase into the winter months and were much higher than oysters sampled from control stations during the period November-March. In addition, the apparent initiation of meat growth with warmer weather occurred several months earlier than at control stations due to elevated temperatures.

Dry weights showed a pattern similar to wet weights. Differences between wet and dry weight patterns were due



to differences in oyster tissue growth and glycogen concentration.

Stations Two and Three showed reductions in dry weight in August. Only at Station One did tissue growth exceed loss of dry weight due to use of reserves and spawning. Since it is impossible to measure dry weights on the same oysters month after month, sampling anomalies must also be considered as possible cause for weight fluctuations.

Dry weight increases during the fall were the result of favorable temperature conditions and high assimilation and growth which lead to tissue growth and glycogen storage. At Station Three in November, oyster dry weights can best be explained as the result of a sampling artifact. Oysters sampled that month were simply larger than the average for that station. The corresponding shell heights and wet weights were both unusually large. Basically, oyster dry weights remained rather low at Oak Orchard throughout the winter and began increasing in March through May. Warmer temperatures during that period and increasing metabolic rate made tissue growth possible.

Oysters from the intake remained near the October-November level until May. Increases in April may be due to tissue growth as active feeding began.

Oysters from Island Creek continued to increase in dry weight well into the winter months due to the extended growing season and the high glycogen concentration in these oys-

ters. Spring decline in dry weight may be due to utilization of reserve glycogen and conversion of it into gonad material. Warming in the effluent in the spring was not as gradual as it was at Stations One and Three and the amount of time the oysters spent exposed to optimum temperature conditions was reduced. Despite the more severe late spring conditions in Island Creek and the possibility that some individuals may have started spawning by the time of the May collection, dry weights were higher there than at the other stations. This shows that throughout the course of this study there has been a net increase in oyster meat weight as well as shell height. Since glycogen concentration has been reduced due to increased metabolic rate, gametogenesis, and gonad formation, it cannot account for increased dry weight.

From a commercial point of view, it is the dry meat weight of the oyster (tissue weight plus glycogen) which is the marketable item. Island Creek oysters far exceeded oysters from control stations in dry weight during the prime harvesting months (December, January, February).

The biomass and  $C^{14}$  uptake of phytoplankters were determined for several stations near the power plant in a study done by investigators from the Department of Geography and Environmental Engineering of Johns Hopkins University (Richard A. Smith, personal communication). Rates of primary production at the discharge were enhanced when

ambient temperatures were below 22°C ( $\Delta t = 7^\circ\text{C}$ ) and rates were depressed when ambient temperatures rose above this level. When primary production rates were altered, they remained altered as long as temperatures were elevated but returned to normal when the water reached ambient temperatures once again. Only at the very highest ambient temperatures and during intermittent chlorination would a  $\Delta t = 7^\circ\text{C}$  cause a permanent destruction of phytoplankton (i.e.--production rate does not return to previous level when ambient temperature returned).

In support of these findings are Warriner and Brehmer (1966) who reported that during periods of low or moderate ambient temperature, a thermal effluent had the effect of enhancing primary productivity. During hot summer temperatures (above 20°C) an increase in temperature depresses productivity. Thus, in the fall, winter, and spring, the effluent would contain more potential oyster food than the other stations. During the summer, the situation would be reversed. Similar results and supporting data have been presented by many workers. Hamilton et al. (1970) confirmed the work of Warriner and Brehmer, but showed that primary productivity could be decreased up to 90% at the outfall by power plant chlorination. Kullberg (1968) reported that freshwater algal diversity decreased as you move closer to the source of a thermal spring. Fedorov et al. (1968) found that productivity of four species of diatoms and green algae

was consistently higher at 20°C than at 10°C. Steeman and Jorgensen (1968) showed that photosynthesis of planktonic algae was higher at 20°C than it was at 2°C or 7°C. Ukeles (1961) discovered that Isochrysis galbana and Monochrysis lutheri, two important species for shellfish culture, will survive temperatures no higher than 24-27°C, while Chlorella was viable above 30°C. Morgan and Stross (1969) found that with a change in temperature of 8°C between the intake and effluent, carbon uptake nearly doubled when ambient water was 16°C or below. When ambient reached 23°C, however, carbon uptake was tremendously reduced. Yanase and Imai (1969) found temperatures between 23°-39°C to be optimum for four species of algae useful in rearing shellfish. Fox and Moyer (1973) reported that the severity of the effects of an effluent on productivity seem to be proportional to the temperature of the intake water. In contrast with most of these findings, Ruddy et al. (personal communication) described no significant differences in total chlorophyll determinations between intake and outfall when the time of measurement did not coincide with the time of intermittent chlorination. On the basis of the major portion of the available literature, it seems likely that the food concentration available to an oyster in the heated effluent is increased during fall, winter, and spring, and decreased during the warmer summer months.

Bayne and Thompson (1970) found that Mytilus edulis

exposed to temperature stress decreased its oxygen consumption rate and utilized body protein as well as carbohydrates as an energy source. Gilluly (1970) reported increased growth of large mouth bass in a pond receiving a heated effluent in South Carolina. Although Roosenburg (1968) found an extended growing season and growth rate for thermally affected oysters during cold months, he found no beneficial or detrimental effects on an annual basis. Brett et al. (1969) in summarizing the effects of thermal releases on fish stated that when maintained at higher temperatures, more food must be available or body weight will decline. Since oyster growth in the effluent was enhanced, it seems likely that primary productivity in Island Creek has kept pace with the increased metabolic demands of the oyster during most of the year, particularly in the fall, winter, and early spring. On the other hand, low primary productivity during the summer is also very likely.

#### Condition

Percent water is a measure of the condition of an oyster. As percent water decreases and solids increase, condition improves. Ideally the water concentration should be nearly the inverse of the glycogen concentration because glycogen is a stored solid which should decrease percent water as it is concentrated.

In the fall as ambient water temperature declined, the

water concentrations of oysters declined from high summer values to the lower values measured in November.

During the winter, Island Creek oysters were lowest in percent water with control stations higher as might be expected during the colder months. The relatively high level of these winter readings at all three stations is unexpected.

At Stations One and Three the water concentration is near the summer level from December through March. At Island Creek, percent water levels were high in December and January. Many factors influence the condition of an oyster. Perhaps the combined effects of several physical parameters combined to affect the condition of the oysters sampled. Galtsoff (1964) showed percent water measurements of nearly 90% for oysters at various times of the year. This mid-winter increase in percent water may reflect a utilization of some stored solids during the period of cold weather inactivity.

In spring, as water temperatures increased, Stations One and Three maintained good condition while the condition of oysters from Island Creek began to decline as glycogen was depleted.

Glycogen is animal starch stored in the connective tissue of the mantle and labial palps of the oyster. C. virginica and many other molluscs show a glycogen cycle, concentrating it in the colder months and using it in gonad

formation as temperatures become warmer. Annual glycogen cycles have been described for various populations of oysters by Galtsoff et al. (1947), Hopkins et al. (1954), and Lee et al. (1960).

Glycogen increased from summer levels into the fall. By August, after spawning, oysters at Island Creek had used some of this reserve glycogen. The control stations were already making a recovery from glycogen depletion due to spawning. Recovery of Island Creek oysters lagged behind that of other stations because warmer temperatures continued into the fall at the effluent station. By December, Island Creek oysters had surpassed control oysters in glycogen concentration. In December, oysters from Stations One and Three showed a decline in glycogen which implies a loss of condition. This is similar to the increase in percent water which occurred at all stations in December, but is of shorter duration.

In April and May, oysters from Island Creek began to decline in glycogen due to gametogenesis and metabolic use of reserves. The late recovery and early loss of condition in Island Creek shows the effects of the shortened winter on oysters held there. Thermal stress is removed last and applied earliest to oysters in Island Creek.

Within a sample of oysters, wet and dry weights and percent water must agree because these measurements are all taken from the same oysters. Glycogen determinations, how-

ever, are necessarily made on different individuals from the sample. Since oysters vary tremendously in many features, this may lead to conflicting data. In reality, percent water and glycogen concentration of a population of oysters cannot both increase in a given month without degeneration of the oyster tissue. Galtsoff (1964) described cases where glycogen and percent water both increase markedly, but offers little in explanation of this phenomenon. Small inconsistencies (August and February, Stations Two and Three; April, Stations One and Three; and all stations in March) can be attributed to sampling artifacts; but larger discrepancies are more difficult to explain (December, Station Two; January, all stations).

Engle (1957) found that optimum condition (percent solids) of oysters occurred in late fall and late spring with a major low through the summer, especially following spawning and a second low in mid-winter. This agrees very well with the percent water data presented here. Haven (1962) found a similar trend using a condition index for oysters held in trays. He also stated that heavy fouling and presence of Polydora websteri tends to be detrimental to condition. Herrmann (1968) studied percent solids of the oyster, Crassostrea gigas, and related seasonal changes with nutrient sources. He found glycogen highest in late spring and lowest in summer, while percent solids was lowest in winter, highest in summer, with a pronounced drop



following spawning. The periods of best condition (spring and fall) were correlated with times of natural phytoplankton blooms.

Widdows and Bayne (1971) stated that the mussel, Mytilus edulis, during warm acclimation showed an increase in blood sugar prior to energy equilibrium suggesting the mobilization and utilization of stored energy reserves. This would serve to explain the low summer glycogen levels of oysters held in Island Creek. The oyster, like Mytilus, was probably unable to maintain its energy balance when first subjected to a temperature increase. Quick (1971) provided supporting evidence and stated that oyster condition declined initially before stabilizing when they were subjected to temperatures of 35°C. Burklew (1971) showed that the higher the exposure temperature, the more rapidly glycogen reserves of the oyster were consumed. He also pointed out the differences in glycogen cycle between northern and southern populations of C. virginica. Our oysters held in the effluent showed a pattern typical of southern oysters concentrating glycogen throughout the fall and winter. Northern populations typically show a mid-winter decline in glycogen during the winter period of inactivity. Oysters at control stations showed this trend to a small degree with a small decline in December. In support of our findings are Ruddy et al. (unpublished data) who measured total carbohydrates of oysters and found that condition was

enhanced in oysters held in the effluent between January and May. The condition indices of oysters sampled from the effluent were higher than those for control oysters in February-May. Total carbohydrates of control and test oysters were similar during summer months. Roosenburg (1968) found no significant differences in condition between oysters held in an effluent and those held in ambient water using an index of condition.

### Spawning

As water temperatures rise in the spring, temperatures which will support gonad formation and spawning are reached first in the effluent. As the water temperatures increase, gonad development progresses and glycogen reserves are consumed (Loosanoff, 1942). When a critical temperature is reached, spawning occurs (Nelson, 1928).

Results indicate that this process occurred first at Island Creek. Oysters from the effluent were successfully spawned on May 7, 1970, whereas oysters from the other stations could not be induced to spawn. Arndt (1968) working in a heated effluent in Maine determined that spawning occurred in the effluent in an area where ambient temperatures were too cold to allow gonad development and spawning to occur. In contrast, Roosenburg (1968) working in Maryland showed no early gonad development in the effluent. He did show a loss of condition of oysters in the effluent

which was interpreted as possible early spawning, but this was not confirmed by gonad examination. Ansell et al. (1964) found an earlier build-up of spawning potentiality and a greater number of eggs produced in clams held in a thermal effluent. Ansell (1963) stated that effluents may be responsible for allowing Mercenaria mercenaria to become established in British waters by raising water temperatures high enough to allow spawning to occur. Ansell and Lander (1967) reported an unusual response of the hard clam to heated effluent. Individuals were found to spawn in both spring and autumn instead of the normal single annual period of spawning. Gennette and Morey (1971) found accelerated spawning of oysters held at 35°C when compared with those held at 30°C.

Confirmation of our tentative results will be necessary using histological examination. If early spawning is shown to have occurred, which we now suspect, it may prove to be a sublethal or chronic effect of the thermal addition. If spawning occurs when ambient water temperatures are below 20°C, larvae will not survive to set and local recruitment may be detrimentally affected. Although the breeding population may not be killed, the population might eventually lose its commercial value or die out altogether.

The use of a thermal effluent in the commercial rearing of shellfish has been suggested by many researchers (Ryther and Bardach, 1968; Ryther, 1969; Ansell, 1969;

Nash, 1970). Methods of increasing the productivity of these estuarine waters have been suggested by Mihursky (1967), Isaacs and Schmidt (1969), and Commercial Fisheries Review (1971). Butler (1953b) found that Chesapeake and Gulf oysters both require 30 months to reach commercial size. Chesapeake oysters are inactive for six months of this period. A thermal effluent might be used in this latitude to promote optimal growth during this period of cold weather inactivity. Based on raft culture data on C. virginica in Delaware Bay (Aprill and Maurer, unpublished data), it is likely that two growing seasons (18 months) would be sufficient time to raise marketable oysters using artificially reared spat and a thermal effluent.

## SUMMARY

Oysters held in the thermal effluent showed shell growth comparable to oysters at control stations throughout the normal growing season. In addition, there was an extension of the growing season into the winter months for oysters held at Island Creek. Meat weights of thermally affected oysters were low in the summer months and mortalities were high due to thermal stress and related factors. Oyster condition based on glycogen reserves was generally poor during the summer and good during cooler weather, with a decline in mid-winter due to the cessation of feeding. Oyster condition reached the extremes at Island Creek, being worst of all stations during the summer months and best during the cooler months. Dry meat weight is the commercial item in oyster production. Oysters from the effluent station were highest in meat weights throughout much of the study, especially during the prime harvesting months. The oyster seems to be resistant to thermal stress and this may be true for other shellfish as well.

In comparing the results of this project with those of Roosenburg (1968), it seems likely that the salinity and turbidity gradient of the Patuxent River, Maryland, may

have masked any differences in growth, condition, or spawning which were due to temperature. As a result of this, Roosenburg found no significant differences between ambient and test stations except in initial mortalities and copper concentration in oyster tissues.

As long as most of our electrical energy is produced by fossil fuel and nuclear power plants, thermal pollution will remain a problem which is constantly growing in magnitude. The effects on our lakes, rivers, and estuaries can be minimized by the use of cooling towers or lagoons. When possible, the site selections for new power facilities on estuaries should be chosen so as to avoid the habitats of commercially and ecologically important species such as the oyster. Where thermal additions are unavoidable, productive uses for waste heat should be sought.

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APPENDIX



APPENDIX A

Group One Oysters

TABLE A-1

Group One  
Mean Monthly Percent Mortality (%)

Station	1	2	3
June	12.7	38.4	30.2
July	3.8	12.5	9.3
August	8.8	13.3	12.7
September	3.4	2.6	3.4
October	1.0	1.6	2.1
November	0.0	1.4	0.0



TABLE A-2

Group One  
Mean Monthly Shell Height (cm)  
Oysters subsampled from those taken from Murderkill River  
June 1970--6.6

Station	1	2	3
August	7.5	7.6	7.6
September	7.5	7.9	8.0
October	8.1	8.0	8.6
November	7.5	8.6	8.2

TABLE A-3

Group One  
Mean Monthly Shell Length (cm)  
Oysters subsampled from those taken from Murderkill River  
June 1970--4.7

Station	1	2	3
August	5.2	5.2	5.3
September	5.4	5.5	5.7
October	5.6	5.5	5.8
November	5.2	5.8	5.8

TABLE A-4

Group One  
Mean Monthly Shell Width (cm)  
Oysters subsampled from those taken from Murderkill River  
June 1970--2.0

Station	1	2	3
August	2.1	2.3	2.2
September	2.3	2.3	2.4
October	2.3	2.2	2.5
November	2.2	2.5	2.5

TABLE A-5

Group One  
Mean Monthly Shell Thickness (cm)--Left Valve  
Oysters subsampled from those taken from Murderkill River  
June 1970--.30

Station	1	2	3
August	.32	.32	.29
September	.31	.33	.31
October	.33	.33	.33
November	.34	.34	.34

TABLE A-6

Group One  
Mean Monthly Shell Thickness (cm) Right Valve  
Oysters subsampled from those taken from Murderkill River  
June 1970--.26

Station	1	2	3
August	.28	.29	.30
September	.28	.30	.32
October	.32	.32	.35
November	.31	.32	.31

TABLE A-7

Group One  
Mean Monthly Wet Weight (g)  
Oysters subsampled from those taken from Murderkill River  
June 1970--5.3

Station	1	2	3
August	5.6	5.1	6.2
September	5.8	6.0	7.9
October	6.0	7.4	10.5
November	6.0	10.9	9.0

TABLE A-8

Group One  
Mean Monthly Dry Weights (g)  
Oysters subsampled from those taken from Murderkill River  
June 1970--1.0

Station	1	2	3
August	1.2	1.1	1.1
September	0.8	1.3	1.5
October	1.1	1.6	2.7
November	1.2	2.8	2.4

TABLE A-9

Group One  
Mean Monthly Percent H<sub>2</sub>O (g)  
Oysters subsampled from those taken from Murderkill River  
June 1970--81.1

Station	1	2	3
August	79.4	79.0	81.9
September	86.7	78.4	80.9
October	81.4	78.1	74.2
November	80.7	74.9	73.4



TABLE A-10

Group One  
Mean Monthly Glycogen  
(mg glycogen/100 g oyster tissue)  
Oysters subsampled from those taken from Murderkill River  
June 1970--1747.6

Station	1	2	3
August	2672.9	1900.4	3880.0
September	3410.6	2341.1	4590.8
October	4287.4	2691.9	5936.3
November	4459.2	4801.0	5680.0



APPENDIX B

Group Two Oysters

TABLE B-1

Group Two  
Mean Shell Length (cm)  
Oysters subsampled from those taken from Murderkill River  
June 1970--4.7

Station	1	2	3
August	5.4	5.2	5.1
September	5.4	5.4	5.2
October	5.7	5.7	5.5
November	5.5	5.4	5.7
December	5.4	5.7	5.0
January	5.5	5.7	5.1
February	5.5	5.7	5.1
March	5.4	5.7	5.3
April	5.5	5.8	5.3
May	5.8	6.4	5.5

TABLE B-2

Group Two  
Mean Shell Width (cm)  
Oysters subsampled from those taken from Murderkill River  
June 1970--2.0

Station	1	2	3
August	2.2	2.1	2.0
September	2.2	2.3	2.1
October	2.3	2.4	2.2
November	2.4	2.5	2.5
December	2.4	2.6	2.2
January	2.3	2.4	2.2
February	2.3	2.5	2.2
March	2.3	2.5	2.4
April	2.5	2.6	2.4
May	2.5	2.8	2.5

TABLE B-3  
Mean Shell Thickness (cm)--Left Valve  
Oysters subsampled from those taken from Murderkill River  
June 1970--.30

Station	1	2	3
August	.32	.32	.29
September	.32	.33	.30
October	.33	.33	.33
November	.34	.34	.34
December	.35	.34	.34
January	.35	.33	.32
February	.35	.33	.32
March	.32	.33	.36
April	.34	.35	.35
May	.35	.35	.34

TABLE B-4

Group Two  
 Mean Shell Thickness (cm)--Right Valve  
 Oysters subsampled from those taken from Murderkill River  
 June 1970--.26

Station	1	2	3
August	.28	.29	.27
September	.29	.31	.28
October	.30	.30	.30
November	.29	.32	.31
December	.32	.32	.30
January	.29	.30	.29
February	.29	.31	.29
March	.28	.29	.31
April	.31	.33	.29
May	.31	.32	.31

