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MARICULTURE RESEARCH AT HENDERSON INLET FOR THE YEAR 1974

ANNUAL REPORT

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SALMON CULTURE PROJECT

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INTRODUCTION

Mariculture site evaluations were conducted during 1973 at Grays Harbor, Willapa Bay, and Henderson Inlet, Puget Sound. Trial pen-rearing studies of salmon at each site indicated Henderson Inlet to be the best suited for a commercial-sized mariculture venture [Snyder, et al, 1974; Didier, 1974 (for citations see Literature Cited Parts I, II, and II)]. As a result of the success of the 1973 studies, the Weyerhaeuser Company entered into a cooperative agreement for the 1974 season with the State of Washington Department of Fisheries and the Fisheries Research Institute:

The State of Washington Department of Fisheries agreed to

1) furnish 80,000 coho salmon and 60,000 chinook salmon for the pen-rearing studies;
2) provide pond space and the tagging trailers which were used for the inoculation of the salmon;
3) conduct its own rearing program at the site which included 92,000 coho salmon and 92,000 chinook salmon.

The Weyerhaeuser Company agreed to

1) supply all manpower and services for the rearing of all fish;
2) provide funds for the mariculture research;
3) assist in monitoring all relevant physical and biological parameters.

The Fisheries Research Institute agreed to

1) assess the environmental impact of a commercial-sized salmon mariculture project;
2) continue disease research on Vibrio sp. to include the following:
    a) effectiveness of intraperitoneal inoculation with vaccines prepared from Henderson Inlet isolates;
    b) monitor all production fish for symptoms of other diseases and blood antibody levels;
3) assess the possibilities of polyculture of invertebrates such as shrimp, mussels, and oysters with salmon.

The University's floating research barge, the R/V Kumtuks, was used as an overall base of operations as well as a laboratory.

Bruce P. Snyder

PART I

SOME EXPERIMENTS WITH *VIBRIO ANGUILLARUM* IMMUNOTHERAPY IN PEN-REARED PACIFIC SALMON

FINAL REPORT OF 1974 EXPERIMENTS

by

Ross G. Antipa

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ABSTRACT

Intraperitoneal injections of three types of Vibrio anguillarum bacterin (heat-killed, Formalin-killed, and a combination of heat and Formalin-killed) were tested in chinook salmon (Oncorhynchus tshawytscha) and coho salmon (O. kisutch) to assess the feasibility of preventing vibriosis by immunization in commercial aquaculture. A single injection of V. anguillarum bacterin gave substantial protection to chinook salmon for 6 months post-inoculation (32% mean mortality in all injected groups) in comparison to a control group (85% mortality). Both species of salmon showed the highest survival when treated with the heat-killed V. anguillarum bacterin. Serum antibody titer of V. anguillarum agglutinins was increased by intraperitoneal injection. No relationship was found between mean hematocrit levels and subsequent mortality. V. anguillarum was the pathogen most frequently isolated from moribund chinook salmon, while Aeromonas salmonicida was the predominant organism isolated from moribund coho salmon.

INTRODUCTION

The potential importance of marine fish farming has recently stimulated research oriented toward the prevention and control of fish disease in the marine environment. One such pathogen is Vibrio anguillarum the etiologic agent of vibriosis, which has been frequently documented as a cause of mortality in salmonids and other marine fishes (Anderson and Conroy, 1970; Cisar and Fryer, 1969; Evelyn, 1971; Fryer et al, 1972; Rucker, 1959; Rucker et al, 1953; Wood, 1974).

Vibriosis can be prevented by oral vaccination (Fletcher and White, 1973; Fryer et al, 1972; Hayashi, et al, 1964; Saito, et al, 1964), drug prophylaxis, or environmental manipulation (Ross, 1970; Wood, 1974). On a production basis, little consideration has been given to injected vaccines because of the high cost of handling (Fletcher and White, 1973; Fryer, et al, 1972; Snieszko, 1970). But recent experimentation with automatic pipetting syringes has indicated that large numbers of fish could be injected at a reasonable cost (Anthony Novotny, personal communication). In addition, laboratory experiments have shown that injection of bacterins produce higher humoral antibody levels than oral vaccination (Fletcher and White, 1973; Hayashi, et al, 1964; Ridgway, et al, 1966).

The purpose of this study was to evaluate the effects of three intraperitoneally-administered V. anguillarum bacterins in chinook salmon (Oncorhynchus tshawytscha) and coho salmon (O. kisutch) subject to a natural disease challenge under commercial aquaculture conditions. For additional information, consult the Ph. D. thesis "Field testing of vaccines in pen-reared Pacific salmon" to be completed by the author by June 1976.

MATERIALS AND METHODS

Vaccine Preparation

A virulent V. anguillarum (HI-163) that was isolated from a chinook salmon held at Henderson Inlet was used to prepare the vaccine. This isolate has been described by Didier (1974) and its identity has been confirmed by DNA homology (Erling Ordal, personal communication).

Test and immunizing antigens were prepared by inoculating 3% Trypticase Soy Broth (BBL) containing a total of 1.5% NaCl and .014% Antifoam A spray (Dow Corning) with a seed culture of V. anguillarum cells. Bacteria were grown for 24 hr at 23 C on a Virtis fermenter at 200 rpm with 18 slpm aeration. The pH was maintained at 7.2 with 1N NaOH. The cells were harvested by centrifugation at 23,500 g (4 C). The bacteria were washed twice with sterile .85% saline, recentrifuged, and finally resuspended in physiological saline at a concentration of 6.7 mg dry wt bacterial cells/ml (23.9 mg wet wt bacterial cells/ml).

Bacteria were killed either by 1) heat (boiling water bath for 1 hr) or 2) Formalin (1% for 24 hr at room temperature). Killed bacteria were

washed, resuspended in sterile physiological saline, and stored at -20 C.

Test Fish

Eight thousand zero-aged chinook salmon (4 g each) were supplied by the Washington State Department of Fisheries Hood Canal Hatchery and the yearling coho salmon (22 g each) were obtained from the Green River Hatchery of the Department.

Vaccination Procedures

Automatic pipetting (1-ml Cornwall) syringes with 26-gauge (9.5 mm long) stainless steel needles were used to inject vaccine. "Tygon" tubing was used to supply the automatic syringes from flasks holding the vaccine stock solution. The bacterin was kept in constant suspension during injection with magnetic stirrers. Temperature of the vaccine suspension was regulated with water and/or ice baths to approximate ambient water temperatures.

Salmon were anesthetized with a 50-ppm concentration of tricane methane-sulfonate (MS-222), hand held, and injected intraperitoneally with vaccine. Skilled workers can inject between 600 and 1,000 22-g salmon/hr. The rate of injecting 4-g salmon was approx 20% slower than that for the larger 22-g fish.

The inoculum volume was .1 ml/coho salmon yearling and .06 ml/zero-age chinook salmon. The coho salmon received a dry weight of .67 mg bacteria/fish (2.39 mg wet wt) and the chinook salmon received .40 mg dry wt bacteria/fish (1.43 mg wet wt). All fish were immunized in freshwater hatcheries and held for 1 week post-inoculation in freshwater before transfer to the salt-water rearing site in truck-mounted planting tanks.

Salt Water Experimental Design

The salt-water rearing area was located at the Weyerhaeuser Company log storage site in Henderson Inlet, southern Puget Sound. Eight floating pens of the type described by Moring (1973) were used in the study. Each of the eight groups of experimental salmon was held in 2.4-m x 2.4-m x 2.1-m nylon mesh nets. Coho salmon were held in 9.5-mm mesh (stretched measure) nets, while the chinook salmon were contained in 6.4-mm mesh nets.

The vaccine treatments included: 2,000 zero-aged chinook salmon and 2,000 yearling coho salmon intraperitoneally-injected with heat-killed V. anguillarum bacterin, 2,000 of each salmon species intraperitoneally-injected with Formalin-killed bacterin, and 2,000 of each species intraperitoneally-injected with a mixture of 50% heat-killed vaccine and 50% Formalin-killed vaccine. An additional 2,000 chinook salmon and 2,000 coho salmon served as uninoculated control groups.

During the experiment, growth was measured by two methods: fork length and weight. Sampling of length and weight was conducted at 2-week intervals from April through October 1974.

All fish were fed unmedicated Oregon Moist Pellets (Formula II, Moore-Clark Company, LaConner, Washington). The same feeding rate was used for each group. Coho salmon were usually fed with a rate of 2.5% to 3.0% body weight/day. New feeding rates were computed approx every week throughout the experiment. Chinook salmon were fed between 2.5% and 3.5% body weight/day.

Diagnostic Methods

Random samples of dead fish were examined to determine the probable cause of death. The following standard bacterial identification tests

were used: wet mount and Gram stained specimens of pathologic tissues, colonial morphology and pigment production on Trypticase Soy Agar (BBL), Kovac's oxidase test, oxidative and fermentative utilization of glucose, 0/129 (2,4-Diamino-6, 7-di-isopropyl pteridine phosphate) sensitivity, growth on Trypticase Soy Broth with 3%, 7%, and 10% total NaCl, sucrose fermentation, cellobiose fermentation, arginine decarboxylase activity, and lysine decarboxylase activity.

Booster Experiments

The effects of reinoculating V. anguillarum antigens into previously immunized coho and chinook salmon were studied in two experiments at Henderson Inlet. Antibody production and survival were the parameters investigated.

The methods used in the coho salmon booster experiment are outlined below. Fifty coho salmon yearlings were intraperitoneally injected with heat-killed V. anguillarum vaccine booster 84 days after initial injection. Each fish received .15 ml of vaccine containing 1.01 mg dry weight of bacterial cells (3.59 mg wet wt). The adipose fin was clipped on the fish receiving the booster treatment. The fifty fish used as controls, as well as the treated fish, were held in a 530-gal salt-water aquarium. One week, two weeks, and five weeks after the experiment began, 15 fish from both the control and treated groups were sampled for serum antibody levels. Mortality was monitored on a daily basis in most instances. Thirty fish were sampled from the stock population before the experiment began, in order to determine pre-booster levels of V. anguillarum specific serum agglutinins.

The methods used in the chinook salmon experiment were similar to those described above for coho salmon. The booster was administered 124 days after initial injection. The 75 treated chinook received 0.1 ml/fish of a heat-killed V. anguillarum suspension containing a dry wt of .67 mg bacterial cells (2.39 mg wet wt). The treated chinook salmon were marked by clipping the adipose fin. The treated chinook salmon and the 75 controls were held together in a 530-gal salt-water aquarium. Chinook salmon were sampled before the experiment began (30 fish) 1 week, 2 weeks, and 3½ weeks after booster administration (15 fish from each group at each sampling).

Serum Antibody Titer

Serum agglutinins to V. anguillarum were quantified using standard microtiter procedures. Blood was obtained by severing the caudal peduncle and collecting the blood in microhematocrit tubes. Heat-killed V. anguillarum (HI-163) at a concentration of 5 mg wet wt/ml was used as the antigen in the agglutination tests (0.3% phenol was added as a preservative). After 18 hr of incubation at 23 C, the titer was detected with a 15X dissecting microscope and recorded.

Erythrocyte Volume

Salmon were anesthetized with MS-222 and the caudal peduncle severed for blood collection into heparinized microhematocrit tubes. The capillary tubes were centrifuged for 4 min at 10,500 rpm.

RESULTS

Cumulative mortality averaged 45.2% for all chinook salmon experimental groups (including control groups) and 5.7% in all groups of coho salmon during the 6 months of the experiment (Table 1). The mortality pattern in the chinook salmon differed considerably among treatments, with the control group sustaining the greatest loss (85.4%) while the group treated with heat-killed vaccine had the least (22.3%). Mortality in the coho salmon was distributed approximately equally in all four groups. Both species showed the best survival when treated with the heat-killed vaccine preparation. A Chi-square test showed the cumulative mortality of the four chinook salmon treatment groups to be significantly different at the 99% confidence level.

The distribution of chinook salmon mortality over time is shown in Fig. 1. Both salmon species had low rates of post-injection mortality. Mortality in the 6,000 vaccinated chinook salmon was three fish (0.5%) during the first week following inoculation, while eight (.13%) of the 6,000 vaccinated coho salmon died during this period. A low-grade initial mortality was seen in all groups following transfer to salt water. This initial mortality can probably be attributed to the stress of salt-water adaptation and to the stress of injection and handling. The first major epizootic occurred in June when over 75% of the chinook control group died, while mortality rates in the vaccinated groups did not appreciably increase. Mortality rates increased in all chinook salmon treatment groups during the first week in September.

Table 1. Mortality in experimental groups of coho and chinook salmon during the interval April 24 through October 3, 1974.

	Coho salmon				Chinook salmon			
Treatment	H&F*	F	C	H	H&F*	F	C	H
Initial population	2000	2000	2000	2000	2000	2000	2000	2000
Mortality	105	135	113	100	706	756	1708	445
Percentage mortality	5.3	6.8	5.7	5.0	35.3	37.8	85.4	22.3
Mean percentage mortality	5.7				45.2			
Significance level of chi square statistic	p=.05				p .005			

H&F* = Formalin and heat-killed vaccine preparation

F = Formalin-killed vaccine preparation

C = control (uninoculated)

H = heat-killed vaccine preparation

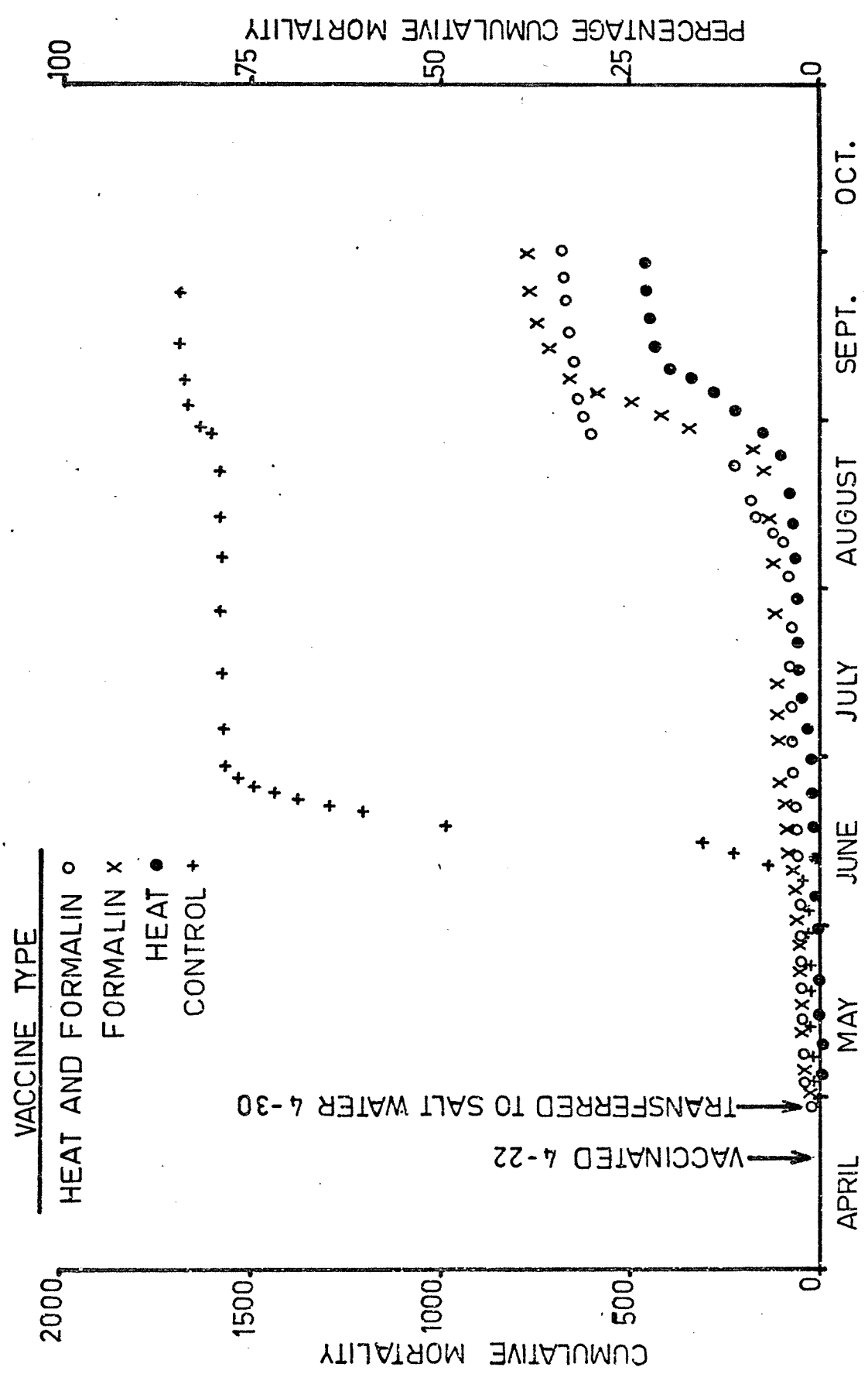

FIG. 1. Cumulative mortality of pen-reared chinook salmon

The mortality distribution in coho salmon over time was similar for each of the four treatment groups (Fig. 2). The only major epizootic occurred during the first 2 weeks of September, which was coincident with increased mortality in chinook salmon.

The etiology of salmon mortalities varied between the two salmon species (Table 2). V. anguillarum was the major cause of chinook salmon mortalities, while coho salmon were more frequently infected with Aeromonas salmonicida. The agent of bacterial kidney disease (Corynebacterium sp.) was also found in both species of salmon. "Other" and "unknown" pathogens did not account for a large percentage of the mortalities examined.

The concentration of chinook salmon serum agglutinins to V. anguillarum rose for all treatment groups (Fig. 3) but the mean antibody level of the control group was usually lower than the level in the injected groups during the first several sampling dates. Antibody levels seen in the chinook salmon control group after the middle of June represent data on the survivors of the epizootic which killed over 80% of that group. The peaks in antibody titer seen in July and September occur about 2 weeks after the two major periods of mortality shown in Fig. 1.

The vaccination of coho salmon resulted in consistently higher anti-V. anguillarum humoral antibody than levels observed in the control group (Fig. 4). The three injected groups of coho salmon showed a rapid increase in antibody titer following injection. The control group showed a much slower increase in titer than the vaccinated groups. After reaching a peak in May (approx 1 month after injection), the titer of the three vaccinated groups began to decline.

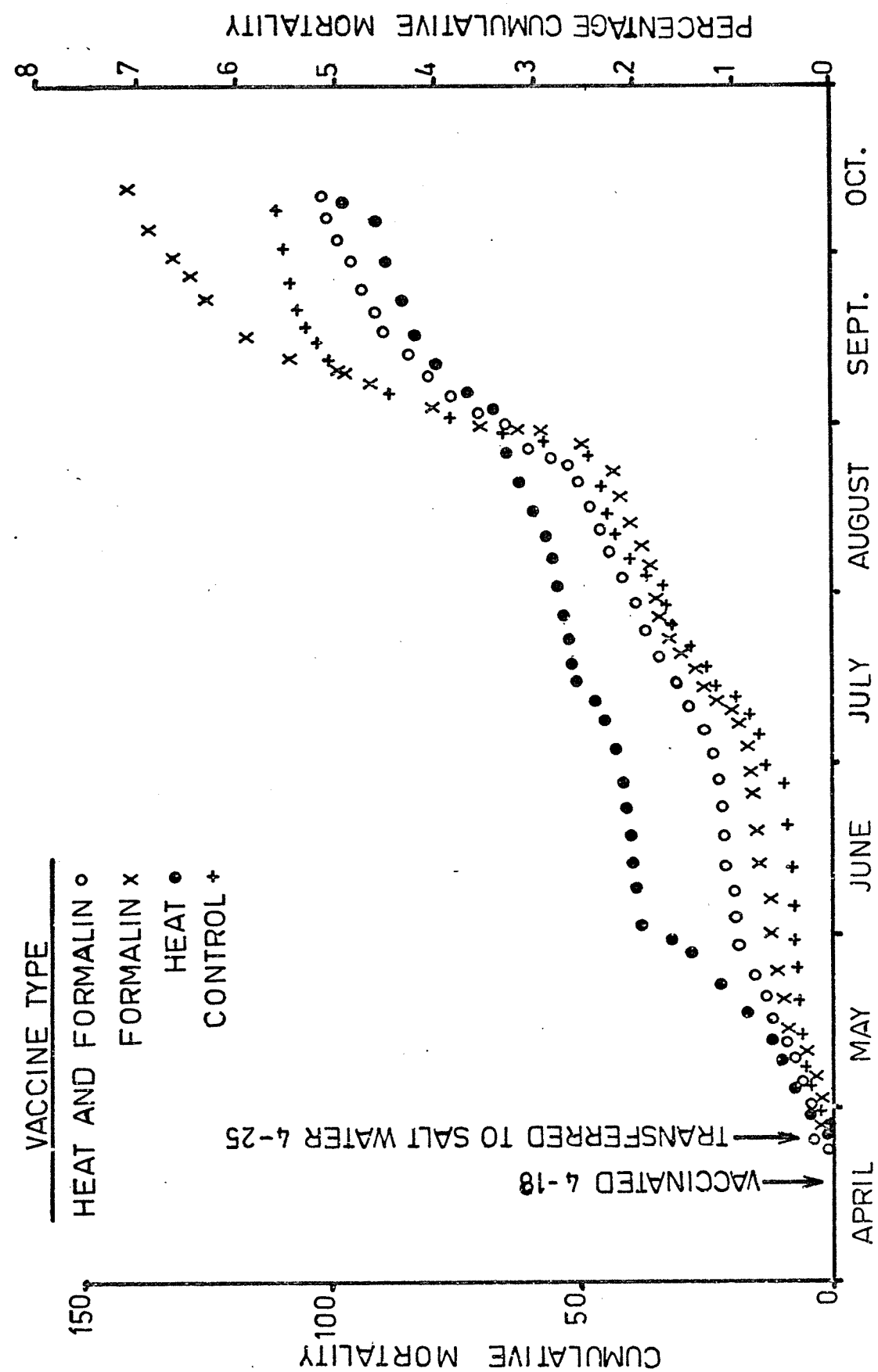

FIG. 2. Cumulative mortality of pen-reared coho salmon.

Table 2 , Bacterial fish pathogens diagnosed from moribund and dead salmon (expressed as a percentage of total isolates positive)

Disease organism	Test fish		Mean
	Chinook salmon	Coho salmon	
Vibrio anguillarum	78.2	36.1	57.2
Aeromonas salmonicida	11.0	41.7	26.4
Corynebacterium sp.	8.3	18.9	13.6
Other	1.6	.2	.9
Unidentified	.9	3.1	2.0

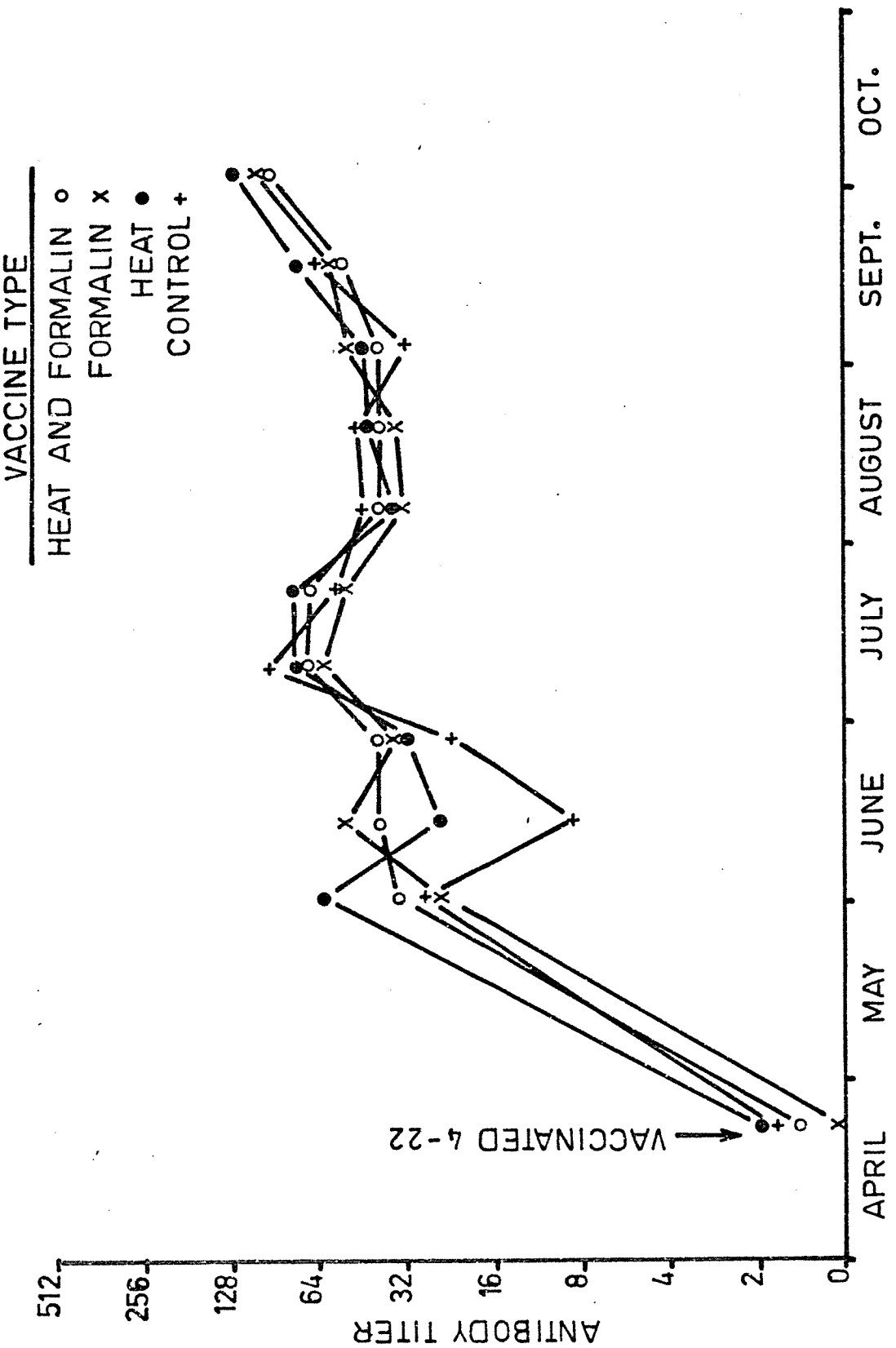

FIG. 3. Mean humoral V. anguillarum agglutinin titer of chinook salmon over time. Each point represents a 20-fish sample.

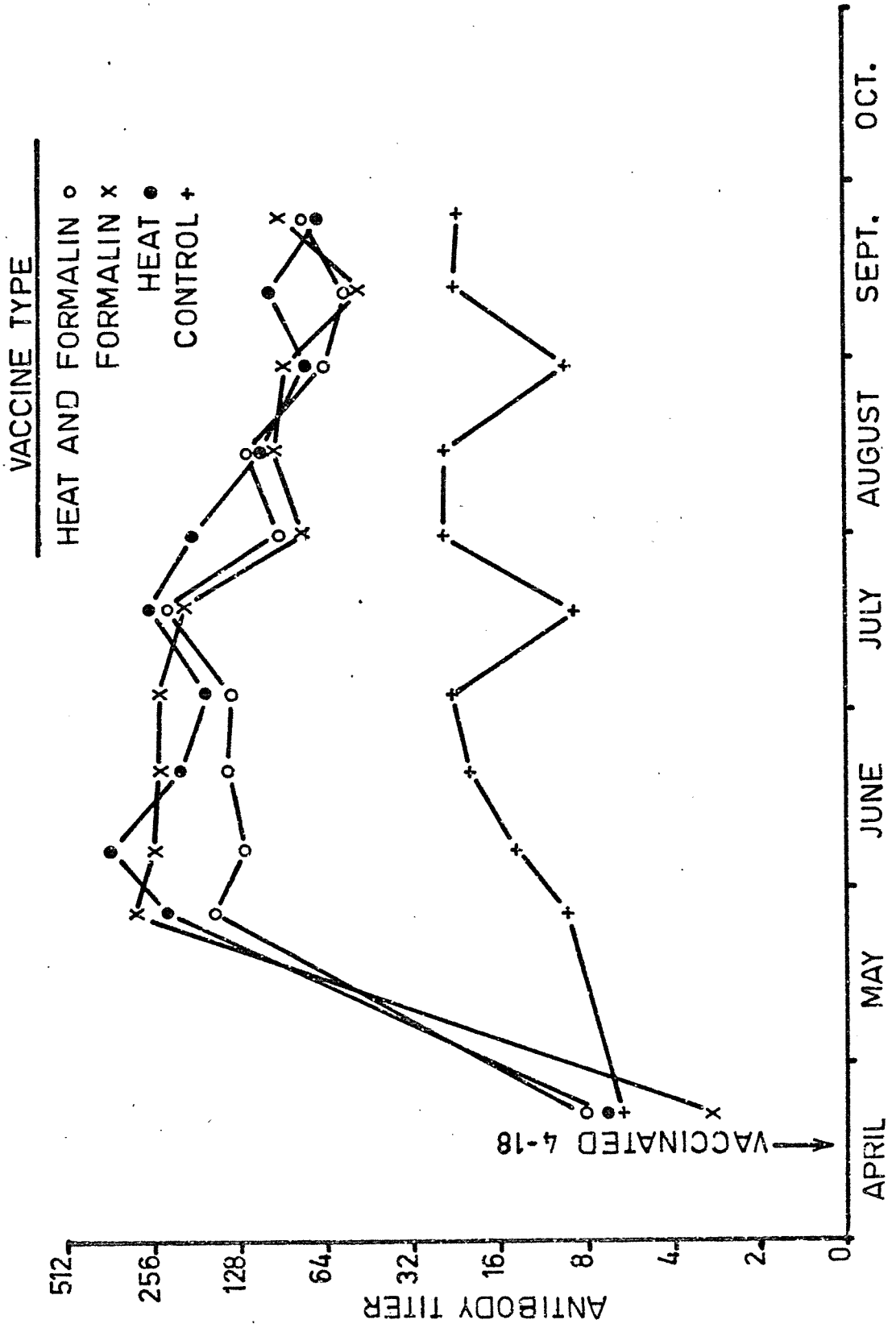

FIG. 4. Mean humoral V. anguillarum agglutinin titer of coho salmon.

Each point represents a 20-fish sample.

The response of coho salmon to a second injection of Vibrio antigen resulted in a higher level of antibody in the treated fish approx 10 days after treatment, when compared with control and pre-treatment samples (Fig. 5). The antibody titer of the experimental group was significantly greater than the control group 2 weeks and 5 weeks after booster treatment, but not after 1 week (one-way analysis of variance, $p < .05$). Antibody levels at the last sampling (5 weeks) were lower than observed 2 weeks after inoculation--thus suggesting a slow loss of agglutinin activity in both the control and treated groups. The lowered titers of both the treated and control group on Week 1 compared to pre-experiment titers may be due to the stress of adaptation to the holding facilities. Water temperatures during this experiment were 15 C $\pm$ 1 C. Disease-caused mortality was 2/50 in the control group and 3/50 in the experimental group.

The chinook salmon responded to the booster treatment by showing increased agglutinin levels (Fig. 6). The antibody titers in the treated group were not significantly different from the control group at the first sampling (Week 1), but they were significantly higher at the second and at the last sampling (one-way analysis of variance $p < .05$). An initial depression of both the control group and treatment group antibody titers below the pre-experiment level was seen at Week 1. These data are similar to the results seen in coho salmon (Fig. 5). Mortality due to disease during the experiment was 21/75 in the treatment group and 32/75 in the control group. Chinook salmon appear to be much more difficult to treat and hold in unnatural surroundings than coho salmon, from the results of this experiment. Water temperatures during the experiment varied from 14.7 C to 15.3 C.

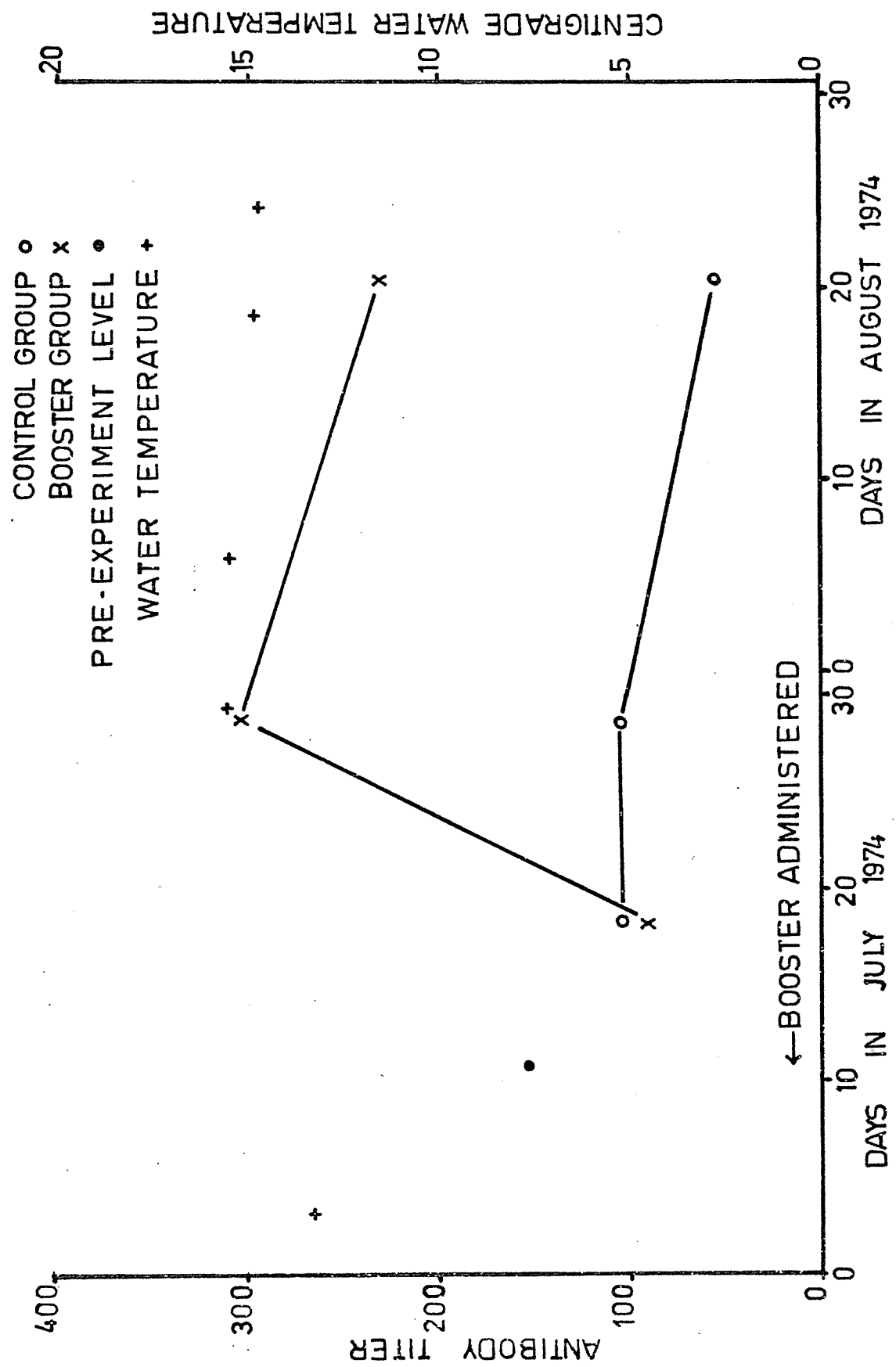

FIG. 5. Booster experiment results: coho salmon antibody vs. time.

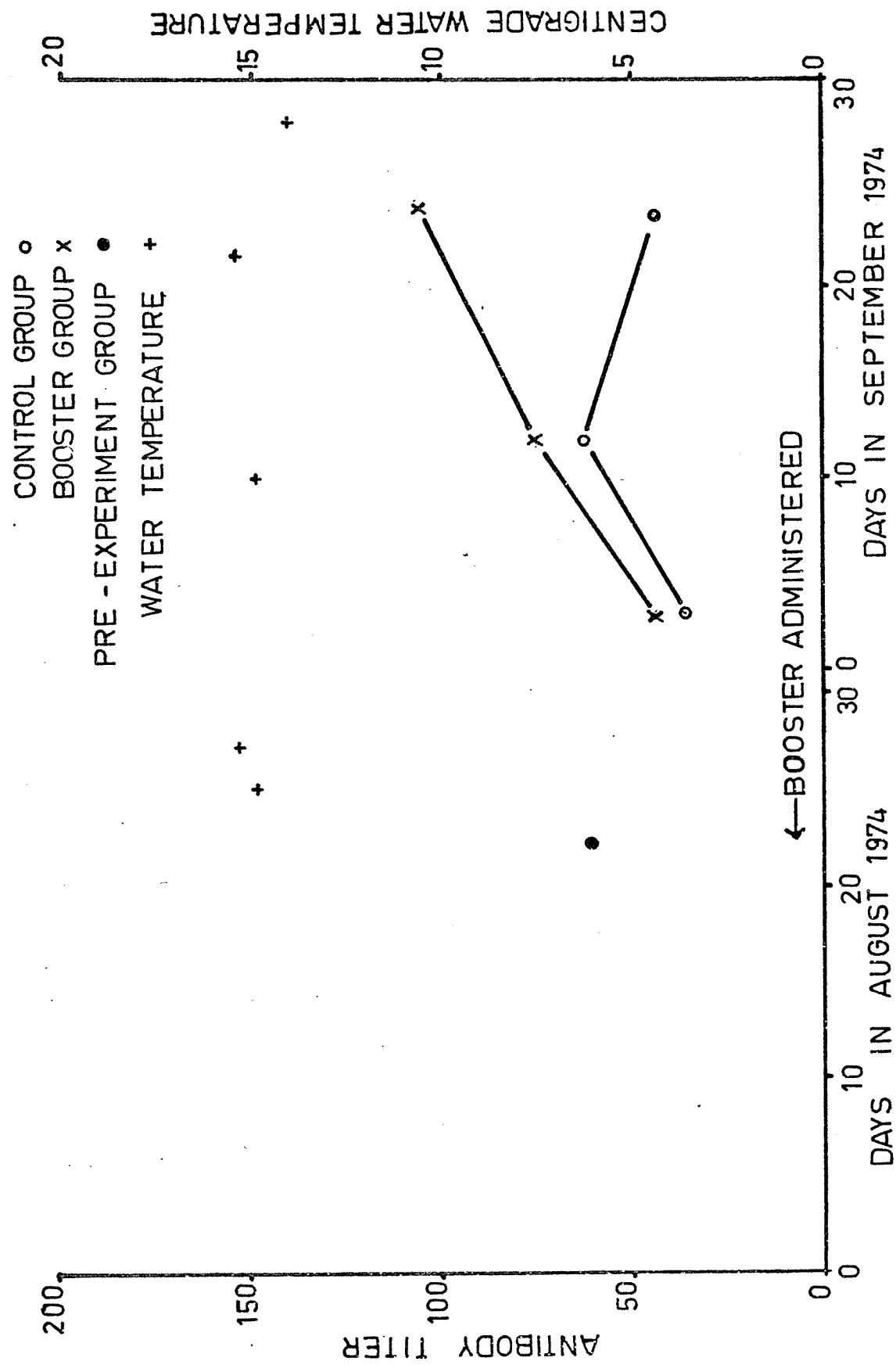

FIG. 6. Booster experiment results: chinook salmon antibody vs. time.

The percentage volume of erythrocytes in the blood (hematocrit) of chinook salmon tended to decline steadily over time (Fig. 7). Significant differences existed between the treatment means on six of ten sampling dates (one-way analysis of variance, $p < .05$) but no consistent patterns emerged.

Coho salmon hematocrit remained relatively constant after an initial decline in May (Fig. 8). No clearcut differences occurred between the hematocrit of the four treatment groups over time. The overall coho salmon hematocrit was 43.3% during the entire study period, an average of 4% higher than the chinook salmon mean hematocrit during the same period.

The percentage volume of leucocytes in the blood plasma of coho salmon tended to increase over time (Fig. 9). The levels seen in the three injected groups are generally higher than those in the control group. The Formalin-treated group and the heat-killed group produced higher values than the other groups. A one-way analysis of variance was used to test the difference between the groups on each sampling date (Table 3). No significant differences ($\alpha = .05$) were found on three sampling dates (June 6, July 2, and July 18, 1974) at the beginning of the sampling period. After July 18, there were significant differences between the treatment groups on each sampling date. The overall mean percentage volume of leucocytes in coho salmon during the experiment was 1.09%.

In chinook salmon, the pattern of percentage volume of leucocytes over time was generally increasing (Fig. 10). The lowest values were usually seen in the control group. The three vaccinated groups vary over time in their respective abilities to stimulate leucocyte production

Table 3. Analysis of variance of coho salmon leucocyte percentage volume data.

Date	Mean Percentage Volume of Leucocytes				1-way ANOVA p
	H&F treatment	F treatment	C treatment	H treatment	
6- 6	.52	.50	.74	.55	.23
6-19	.42	1.62	.55	.39	.00
7- 2	.95	.83	.62	.64	.16
7-18	.71	.46	.73	.62	.14
7-30	.64	1.95	.71	1.13	.00
8-15	1.18	1.46	.92	2.06	.00
8-27	1.30	1.60	.56	1.78	.00
9-10	1.04	1.74	1.44	1.61	.00
9-24	1.54	1.65	1.60	2.37	.00

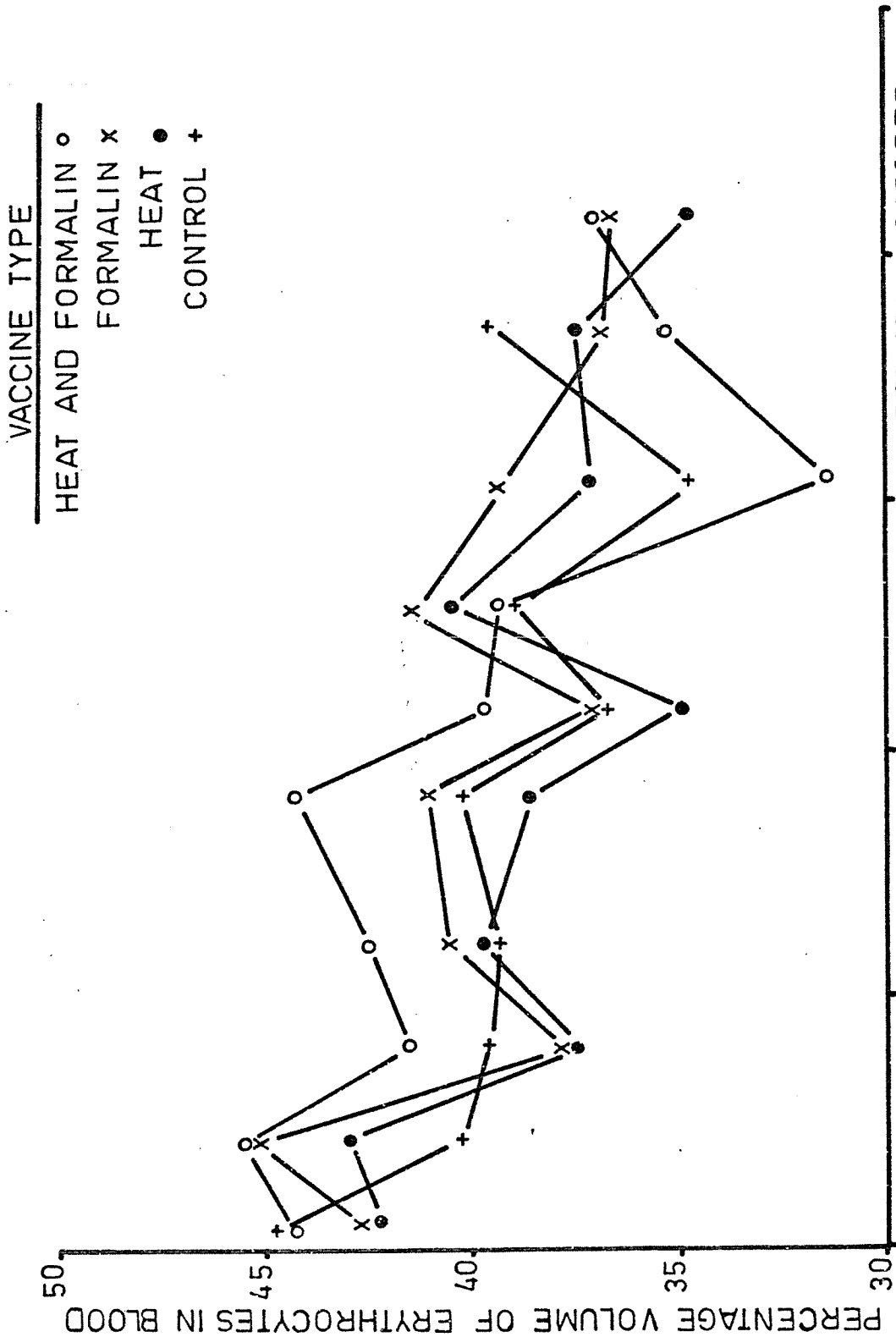

FIG. 7. Hematocrit of chinook salmon. A 20-fish sample mean composes each point.

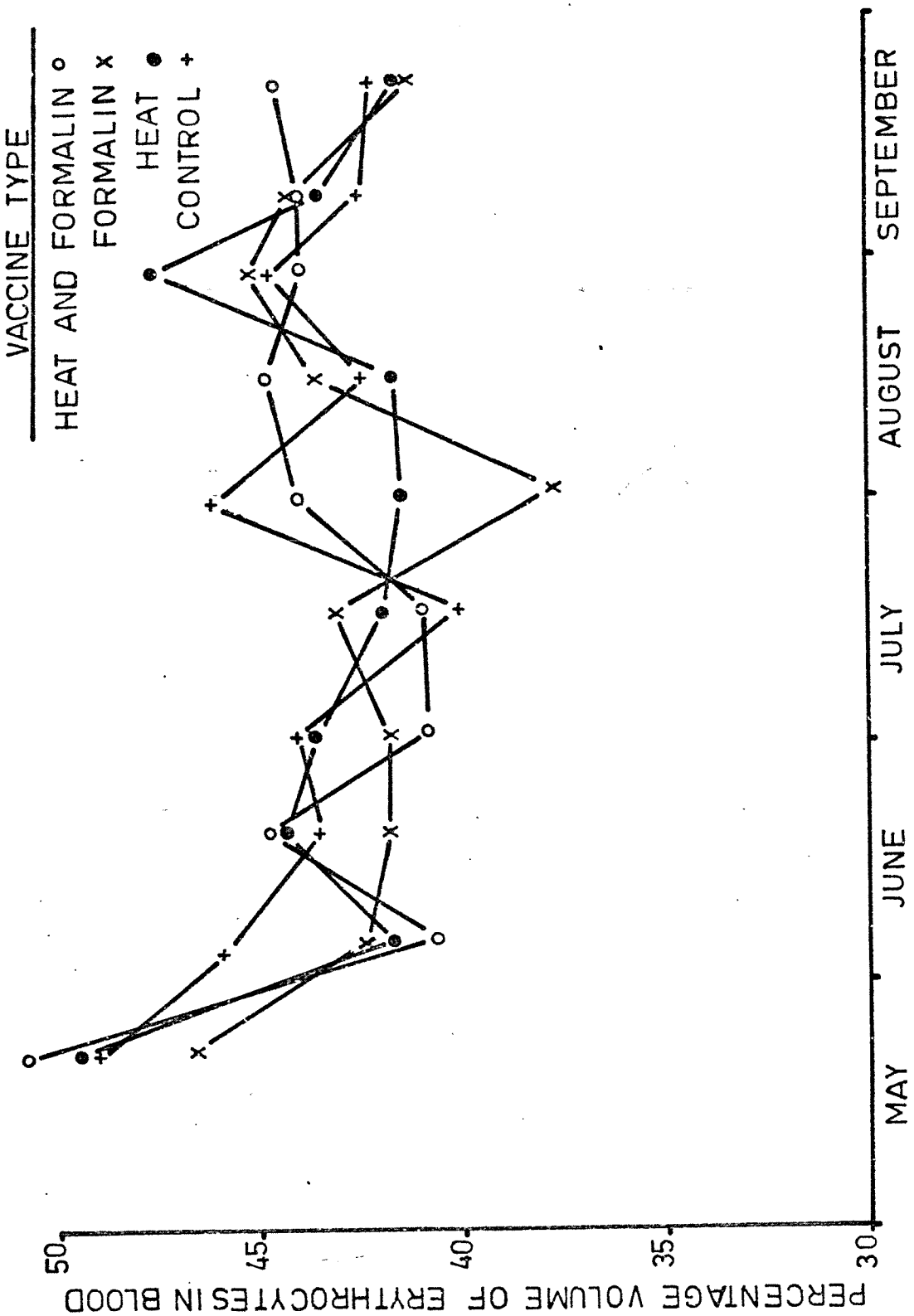

FIG. 8. Hematocrit of coho salmon. Each point represents the mean of a 20-fish sample.

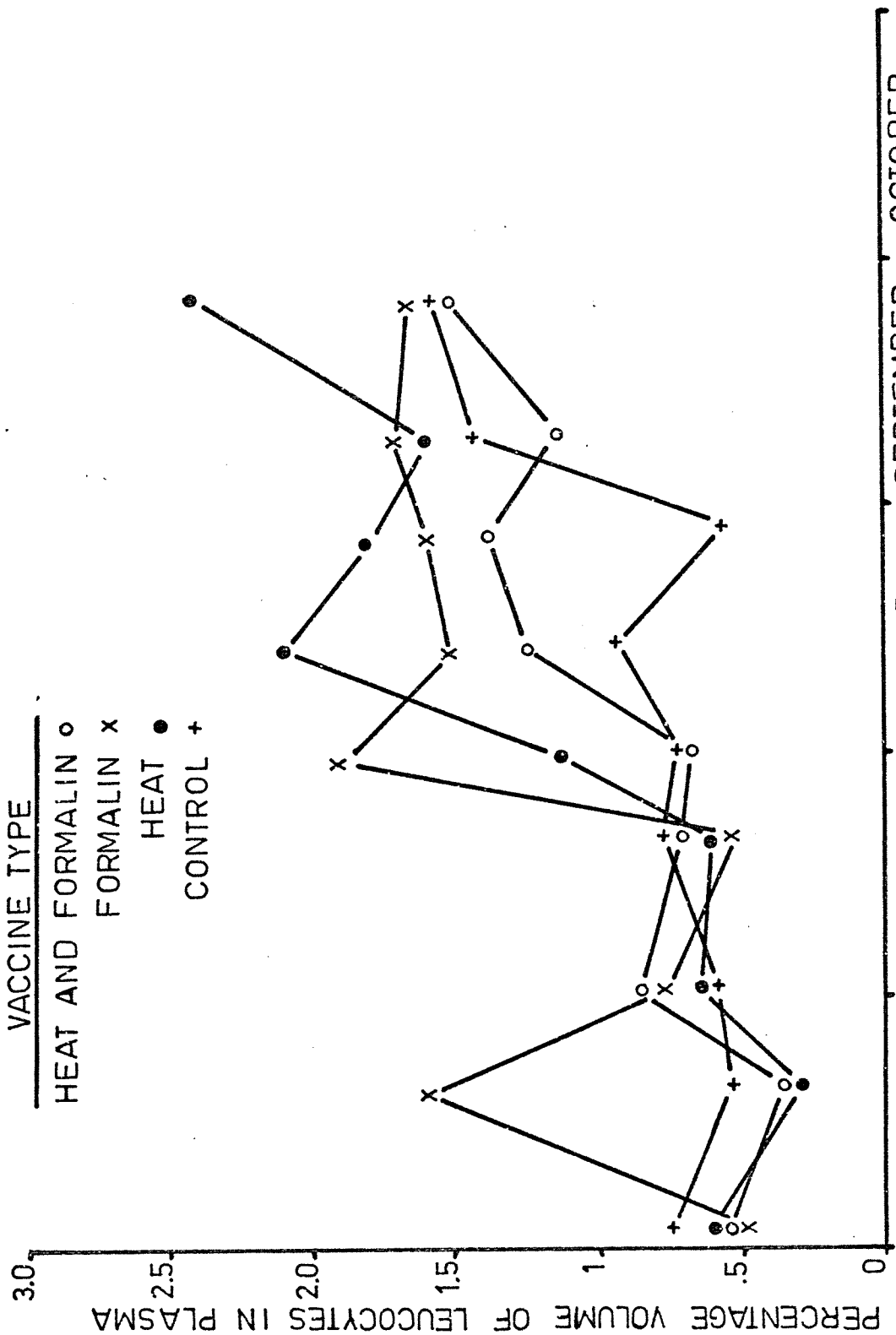

FIG. 9. Coho salmon mean leucocyte volume over time.

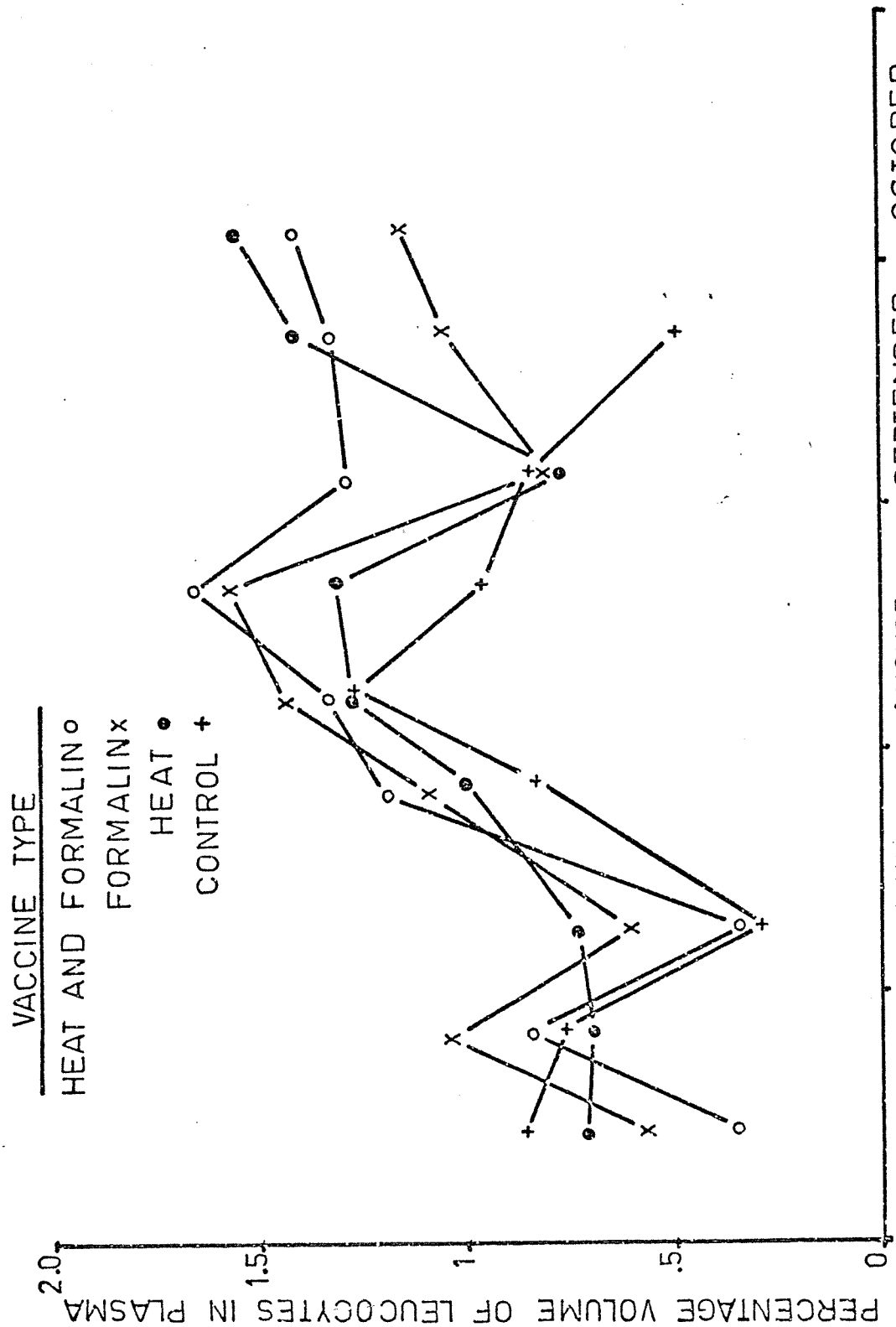

FIG. 10. Chinook salmon mean leucocyte volume over time.

with no one group consistently being higher than the others. The two depresssions in the curve (July and September) are roughly correlated to periods of high mortality. An analysis of variance of these data appears in Table 4. Significant differences occur over most of the sampling dates (June 9), with the control group being usually lower than the three treated groups. The overall mean percentage volume of leucocytes in chinook salmon was .98%, approx 10% lower than the values found for coho salmon.

The relationship of weight over time was very similar for chinook and coho salmon in the 1974 experiment (Fig. 11). The coho salmon were initially larger than the chinook salmon because they were 1 year older, but the pattern of weight gain over time for the two species was otherwise very similar. Growth of the 1973 chinook salmon experimental group was much faster than that of the 1974 chinook, most evident in August and September.

The rate of increase in fork length over time was somewhat greater in chinook salmon than in coho salmon (Fig. 12). The fork length data for both salmon species appears to be linear over time. The length data of coho salmon was analyzed statistically for differences on each sampling date in Table 5. After August 6, coho salmon fork lengths are significantly different between treatments ($p < .05$). This is largely attributable to the morerapid growth of the control group (which had 564 "unknown" losses during the experiment but received the same amount of food as the other pens).

The analysis of chinook salmon fork lengths between pens is shown in Table 6. Significant differences ($p < .05$) in chinook fork length (pen means) was first observed on July 22. The pen means continued to be significantly different until the entire control group died

Table 4. Analysis of variance of chinook salmon percentage volume of leucocytes

Date	Mean Percentage Volume of Leucocytes				1-way ANOVA
	H&F treatment	F treatment	C treatment	H treatment	p
6-12	.38	.59	.78	.69	.01
6-25	.80	1.02	.69	.64	.01
7-10	.29	.55	.27	.70	.01
7-23	1.16	1.04	.71	.98	.06
8- 6	1.32	1.43	1.12	1.25	.52
8-21	1.72	1.57	.95	1.32	.00
9- 4	1.27	.81	.85	.74	.03
9-18	1.30	1.09	.63	1.43	.00
10- 2	1.41	1.13		1.65	.06

Table 5. One-way analysis of variance of coho salmon mean fork lengths over time.

Date	Mean Fork Length				1-way ANOVA p
	H&F treatment	F treatment	C treatment	H treatment	
4-29	119.9	120.8	119.8	119.7	.97
5-15	126.6	127.7	127.3	127.8	.91
5-28	131.6	131.8	130.2	131.8	.75
6-11	136.0	135.3	134.6	134.4	.82
6-25	143.5	141.6	139.4	141.4	.24
7- 9	144.8	149.6	148.5	147.9	.21
7-22	148.7	150.3	154.2	151.2	.19
8- 6	152.6	159.1	160.8	155.0	.02
8-20	164.3	166.3	173.2	166.3	.03
9- 4	173.0	169.7	177.5	171.8	.04
9-17	177.1	178.3	187.1	174.0	.00
10- 3	192.5	185.1	200.7	185.9	.00

Table 6. One-way analysis of variance of chinook salmon mean fork lengths over time

Date	Mean Fork Length				1-way ANOVA P
	H&F treatment	F treatment	C treatment	H treatment	
5- 2	66.1	65.8	66.5	68.0	.07
5-17	74.0	74.3	75.5	74.4	.61
5-28	85.1	84.9	84.1	84.9	.85
6-11	90.5	91.9	93.4	93.4	.12
6-25	102.5	104.3	103.0	104.1	.55
7- 9	112.7	115.6	114.4	111.8	.11
7-22	122.7	128.0	123.6	122.6	.00
8- 6	132.5	141.7	133.8	129.9	.00
8-20	133.4	151.6	138.8	138.9	.00
9- 4	144.3	161.3	147.5	146.2	.00
9-17	151.0	166.9	153.9	155.5	.00
10- 3	167.1		165.5	168.4	.51

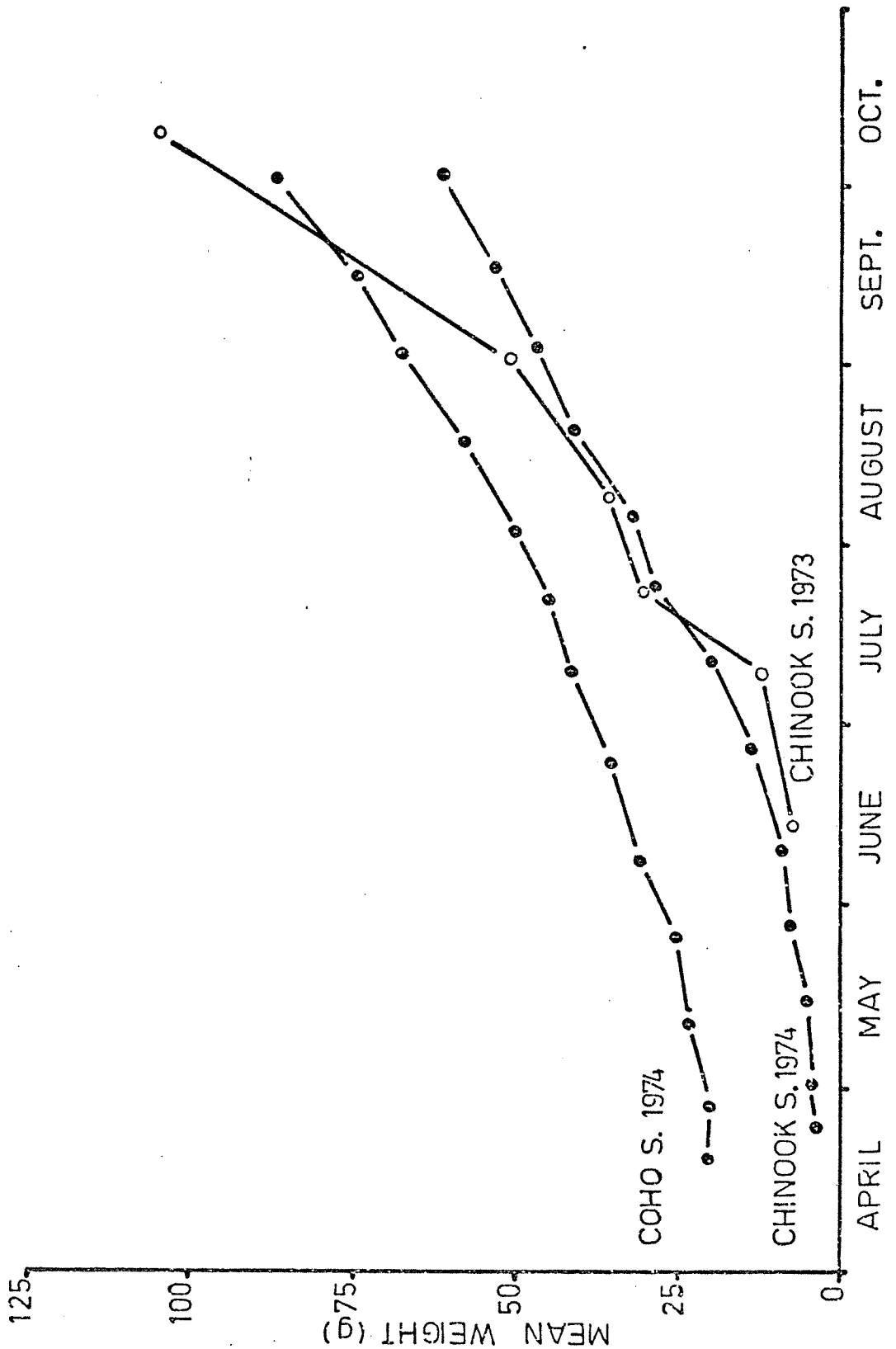

FIG. 11. Mean weight over time for the 1974 experimental chinook and coho salmon and for the 1973 chinook salmon at Henderson Inlet,

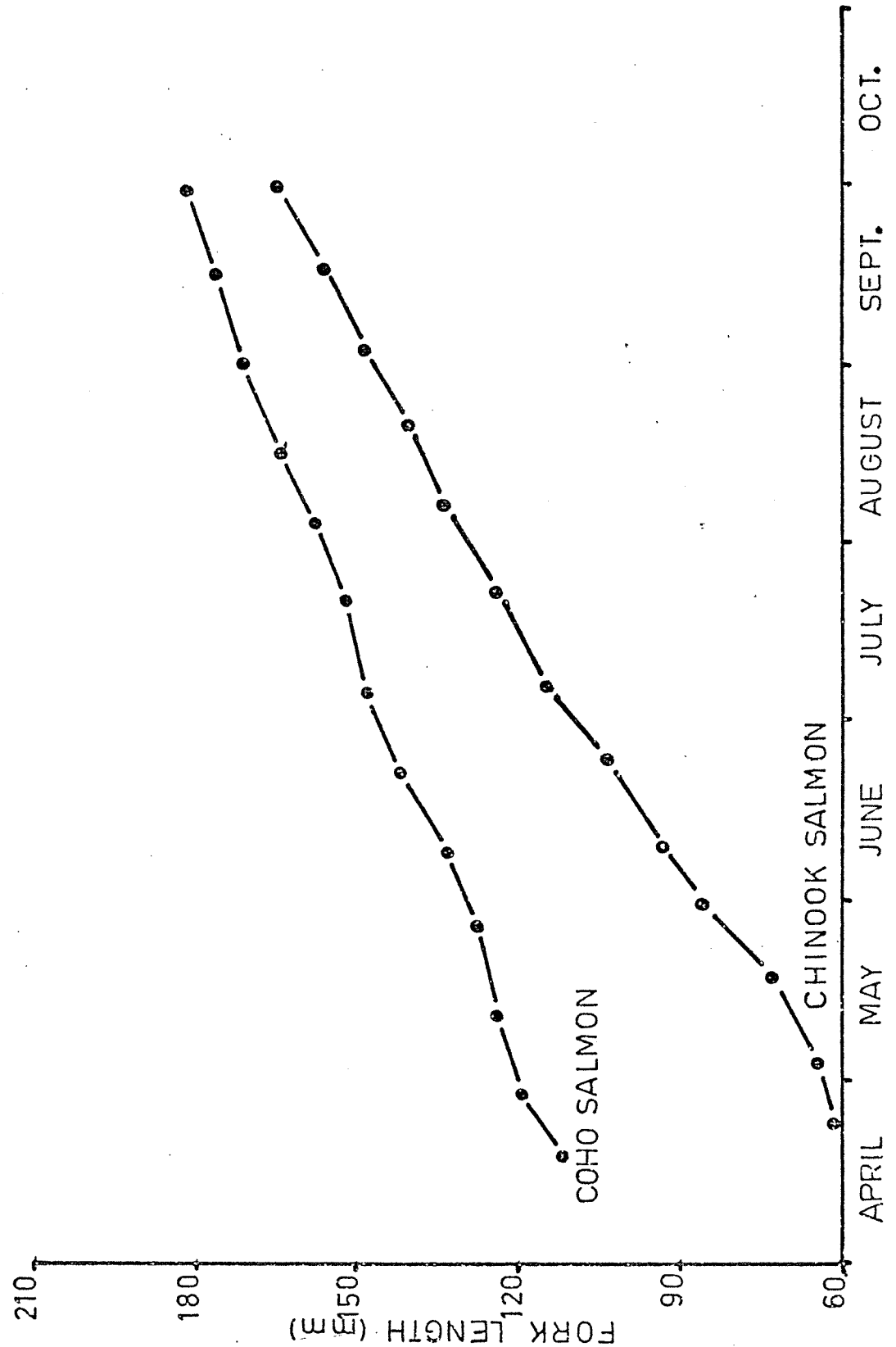

FIG. 12. Mean fork length over time for chinook and coho salmon.

(October 3, 1974). It appears that the faster growth of the control group was the chief factor in the rejection of the hypothesis of equal growth rates in the four treatment groups. Faster growth in the control group is largely due to the high mortality incurred in June that left only a small fraction of the fish alive and to the fact that feeding rates were difficult to equalize with the other groups. Therefore, no conclusions can be drawn on the effects of injection on growth in chinook salmon from this experiment. There appears to be little difference in the growth rates of the three injected groups.

Overall food conversion rates are calculated in Table 7, using the assumption that the sampled fish should be considered as a natural mortality factor. Conversion rates ranged from 1.9 to 2.9 in the seven groups that survived until the experiment was completed. The best chinook salmon group was the heat-killed vaccinated group (1.9), while the heat and Formalin vaccine proved best with the coho salmon (2.0). The vaccination treatment did not appear to lower the food conversion rates of coho salmon in comparison to the control group (the control group of chinook salmon did not survive, making comparisons impossible in this case).

Food conversion rates were also calculated by assuming that the sampling mortalities would have survived until the end of the experiment (Table 8). Overall conversion rates in this analysis ranged from 1.6 to 2.4 (exclusive of the chinook control). The true conversion rates probably lie somewhere between the values in Tables 7 and 8.

The weekly mean water temperature at depths of 0.5 m and 2.0 m is plotted with time in Fig. 13. These data are included because of the

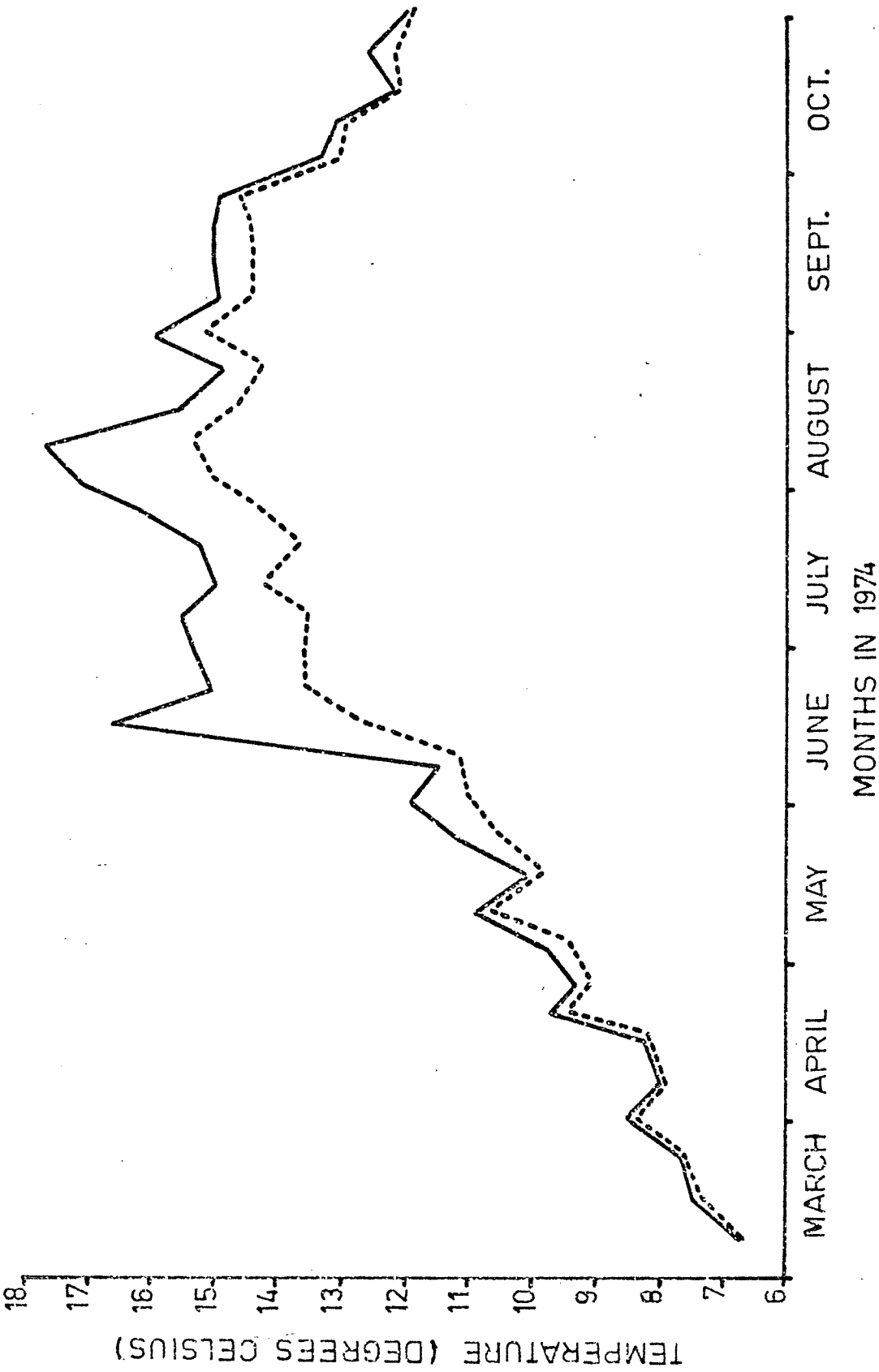

FIG. 13. Weekly mean 0.5 m (solid line) and 2.0 m (dashed line) ambient water temperature at the pen-rearing site.

Table 7. Food conversion data for the period April 29 through October 3, 1974; sampling mortalities are considered a component of natural mortality

Species	Treatment	Population at beginning of experiment		Population at end of experiment		lbs food fed	Conversion rate
		Number of fish	lbs of fish	Number of fish	lbs of fish		
Coho	H&F	2000	82.0	1612	324	476	2.0
Coho	F	2000	82.0	1577	282	476	2.4
Coho	C	2000	84.0	1119	260	476	2.7
Coho	H	2000	80.0	1647	300	476	2.2
Chinook	H&F	2000	15.6	1065	142	325	2.6
Chinook	F	2000	14.0	946	125	325	2.9
Chinook	C	2000	16.4	0	0	87	∞
Chinook	H	2000	15.2	1306	184	325	1.9

Table 8. Food conversion data for the period April 29 through October 3, 1974; sampling mortalities are assumed to have survived to the end of the experiment

Species	Treatment	Population at beginning of experiment		Population at end of experiment		lbs food fed	Conversion rate
		Number of fish	lbs of fish	Number of fish	lbs of fish		
Coho	H&F	2000	82.0	1815	364	476	1.7
Coho	F	2000	82.0	1780	318	476	2.0
Coho	C	2000	84.0	1320	307	476	2.1
Coho	H	2000	80.0	1847	336	476	1.9
Chinook	H&F	2000	15.6	1296	173	325	2.1
Chinook	F	2000	14.0	1146	151	325	2.4
Chinook	C	2000	16.4	202	31	87	3.6
Chinook	H	2000	15.2	1513	213	325	1.6

influence of water temperature on the immune response of fish reported by Muroga and Egusa (1969).

DISCUSSION

A single intraperitoneal injection of V. anguillarum bacterin in chinook salmon resulted in substantial protection against vibriosis for 6 months post-inoculation. Although the effects of stress of intraperitoneal vaccination on fish have not been fully quantified, the mortality incurred during vaccination and several weeks post-inoculation was low in this study. Protection against vibriosis of this magnitude and duration, coupled with minimal adverse effects, indicates that vaccination by injection may have practical application in commercial marine aquaculture. Recent investigations by Rohovec (1975) showed that oral and intraperitoneal V. anguillarum immunization of chinook salmon had about the same effectiveness during a 40-day natural challenge. The injection method of vaccination appears to have some advantages over oral vaccination, such as: the amount of bacterin administered to each fish is more constant, a lesser amount of bacterin is needed to immunize each fish, and no special food processing is necessary. In this study, the cost of administering vaccine by injection was somewhat less than one cent/fish.

The role of humoral antibody in the prevention of natural Vibrio infections is not known. This is complicated by the fact that the invasion route of the bacterium under natural conditions has not been determined. Passive transfer of antibody has shown that serum agglutinins can prevent vibriosis in recipients (Harrell, 1973; Gunnels, 1974), and thus can be important in preventing disease. In this experiment, both

chinook and coho salmon developed serum anti-V. anguillarum agglutinins following vaccination. The appearance of serum agglutinins in control groups of both salmon species is probably due to natural exposure to vibrioids--and may be a mechanism of natural immunity in chinook and coho salmon. The level of serum agglutinins necessary to affect immunity may vary with the intensity of the natural bacterial challenge, the physiological condition of the fish, environmental conditions, and other factors.

In vaccine preparations, the methods used for killing bacteria (heat, sound, or various chemicals) are known to affect the antigenic composition of the bacterin. Heat-killing usually destroys all but the cell wall (somatic) antigens, while Formalin-killing typically preserves the fragile flagellar antigens. However, somatic antigens in the Formalin-killed preparation may not be accessible to the antibody-forming cells of the fish, so a combination of the two bacterins was tested. Although the specific antigen (or antigens) that stimulate the formation of antibodies against V. anguillarum in salmonids is not known, it was proposed that a strong natural disease challenge would select for the fish treated with the most protective bacterin. In this experiment, the heat-killed bacterin proved slightly more effective in both salmon species. These results are similar to those of Harrel (1973) who found that heat-killed Vibrio bacterins were more protective than Formalin-killed preparations in rainbow trout.

Levin, et al (1972) and Cardwell and Smith (1971) showed that fish infected with V. anguillarum typically show depressed packed erythrocyte volume (hematocrit). Hematocrit was monitored in this study to assess

its possible use as a tool to predict V. anguillarum epizootics in large-scale fish culture. Data from this study did not reveal a correlation between low hematocrit levels and subsequent increased mortality. This is probably due to the short latency period (time between infection and the first signs of disease) typical of V. anguillarum infections in salmonids held in warm water (>12 C). Diseases of a more chronic nature may result in depressed hematocrit levels of sufficient duration to be detectable by random sampling from a fish population.

The principal cause of disease-related mortality in chinook salmon was infection with V. anguillarum. In contrast, A. salmonicida was the largest cause of mortality in coho salmon. The difference in incidence of disease organisms in the two salmon species may be related to: variation in species susceptibility to vibrio infections, the longer residence time of coho salmon in freshwater (1 year, compared to several months in the case of chinook salmon), and the origin of the two species of salmon in separate freshwater environments with attendant differences in disease exposure and stress history.

Future testing of potential methods of immunization for use in aquaculture must be accompanied by field tests that span the entire growing period that is subject to significant disease challenge. Short-term natural disease challenge experiments are applicable to aquaculture in only a limited manner. Data from the last 2 years at Henderson Inlet indicate that significant vibrio epizootics may be expected anytime between May and October. Salmon grown for the pan-size market must normally be held during only one summer in salt-water pens,

and thus require protection against Vibrio infections for approx 6 months. The pattern of serum antibody over time in coho salmon (Fig. 4) showed a decrease in all of the vaccinated groups with time. This decline of serum antibody titer over time demonstrates that some aspects of immunization are temporary and that perhaps a booster treatment may be desirable in order to protect the fish through the challenge period. Chinook salmon showed an irregular increase in serum antibody titer over time (Fig. 3) which probably reflects repeated environmental challenge by vibrioids. Coho salmon, on the other hand, showed little evidence of significant natural Vibrio disease challenge during the experiment.

Absolute protection against vibriosis was not achieved in this vaccination experiment but the demonstrated degree of protection against disease does indicate the feasibility for commercial use of intraperitoneal injection for fish immunization. The field of fish immunization is in a state of intense investigation at the present time, and it is difficult to predict what the preferred methods of the future will be. The present method is an economical and effective means of protecting fish against vibriosis.

CONCLUSIONS

Initial mortality due to injection was negligible.

Survival of the three injected groups of chinook salmon was significantly higher than the control group.

Mortality in the coho salmon was low (mean 5.7%) and not appreciably different between the control and treated groups.

The heat-killed vaccine preparation provided the best potential survival in both chinook and coho salmon.

Vibrio anguillarum was the primary cause of mortality in chinook salmon.

Aeromonas salmonicida was the primary cause of coho salmon mortality (41.7%) followed by V. anguillarum (36.1%).

Unknown losses (probably due to escapement through holes in the pen nets) were as high as 28.2% in some groups.

Large-scale mortalities due to V. anguillarum first occurred (June 10) when .5-m-water temperatures averaged 16.3 C (weekly mean) and 2.0-m-water temperatures averaged 17.7 C.

The last major epizootic occurred on September 8 when weekly water temperatures averaged 14.8 C at .5-m and 14.2 C at 2.0 m.

The onset of epizootics was very sudden in most cases---and underlined the need for frequent mortality counts to establish the need for chemotherapy under production conditions.

Injection produced higher antibody titers in the treated groups compared to levels seen in control groups, particularly with coho salmon.

There is some evidence that antibody titers are directly related to chances of survival.

Coho salmon showed a linear decline in antibody titer over time.

Chinook salmon responded with lower antibody levels following injection than coho salmon.

Antibody levels in chinook salmon appeared to rise 2 to 3 weeks after the two major epizootics occurred and did not show the overall linear decline seen in coho salmon.

Administration of an injected booster resulted in increased antibody titer compared to control groups in a small-scale experiment with coho and chinook salmon. A slow loss of agglutinin activity was seen over time, suggesting that boosters could serve as a temporary means of increasing disease resistance.

The average growth rates of chinook and coho salmon were very similar in 1974, and somewhat slower than in 1973.

No conclusions can be made with respect to effects of injection on growth rates because of excessive mortality (with associated density

changes) in the chinook salmon control group and because of escapees in the coho control group.

Food conversion rates in the seven salmon groups that survived the experiment ranged between 1.6 and 2.4 (Table 8), which was comparable to 1973 data from the same site.

After an initial decline, coho salmon hematocrit data tended to reamin constant over time. The hematocrit of chinook salmon declined over time. Differences between the treatment groups were usually not consistent over time in both salmon species.

The percentage volume of leucocytes increased over time in both salmon species; in general, the control groups showed lower levels than the treated groups.

PART II

THE EFFECT OF ORGANIC ENRICHMENT FROM A SALMON MARICULTURE FACILITY ON THE WATER QUALITY AND BENTHIC COMMUNITY OF HENDERSON INLET, WASHINGTON

FINAL REPORT OF 1974 EXPERIMENTS

by

Bruce C. Pease

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ABSTRACT

This is a report on the effect of Weyerhaeuser Company's 1974 experimental mariculture facility at Henderson Inlet on its surrounding marine environment. The study is divided into two major subdivisions: 1) a study of the water quality of Henderson Inlet as related to potential effects of soluble excretion and respiration products from the fish at the mariculture site, and 2) a study of the benthic environment of Henderson Inlet as related to potential effects of the accumulation of relatively insoluble organic material, such as fish feces and excess food at the mariculture site.

The results of this study indicate that the mariculture facility had little, if any, adverse effect on the water quality of Henderson Inlet, which is primarily regulated by phytoplankton activity and the natural hydrographic characteristics of Puget Sound. However, there was a significant accumulation of organic material under the salmon pens, which caused a significant change in the structure of the benthic community.

THE EFFECT OF ORGANIC ENRICHMENT FROM A SALMON MARICULTURE FACILITY ON THE WATER QUALITY AND BENTHIC COMMUNITY OF HENDERSON INLET, WASHINGTON

Final Report for the Period January through December 1974

Bruce C. Pease

INTRODUCTION

Background

Henderson Inlet is a shallow embayment of southern Puget Sound. The Weyerhaeuser Company has operated a 200-acre log dumping and rafting facility on the western shore of Henderson Inlet since 1928. In 1973, Weyerhaeuser began salmonid mariculture as a new venture and Henderson Inlet was one of three sites selected for preliminary studies. These studies, conducted by the University of Washington under contract with Weyerhaeuser, indicated that Henderson Inlet is the most suitable site of the three for further studies (Snyder, Didier, and Salo, 1974). Therefore, all of Weyerhaeuser's 1974 salmonid mariculture research was conducted at Henderson Inlet.

The 1974 mariculture facility was located in a log-raft storage slip on the outer edge of the log dump (Fig. 1). An area of approx 100 ft x 400 ft within the slip was used to grow 250,000 Weyerhaeuser zero-aged chinook and yearling coho salmon from May 1974 to March 1975, with 98,000 yearling coho salmon held in the slip area from April to July 1974 for the Washington State Department of Fisheries release program. The University held 16,000 zero-aged chinook and yearling coho salmon in the slip area for a disease study. A University research barge, the R/V Kumtuks, was used as a support facility within the slip area. Most

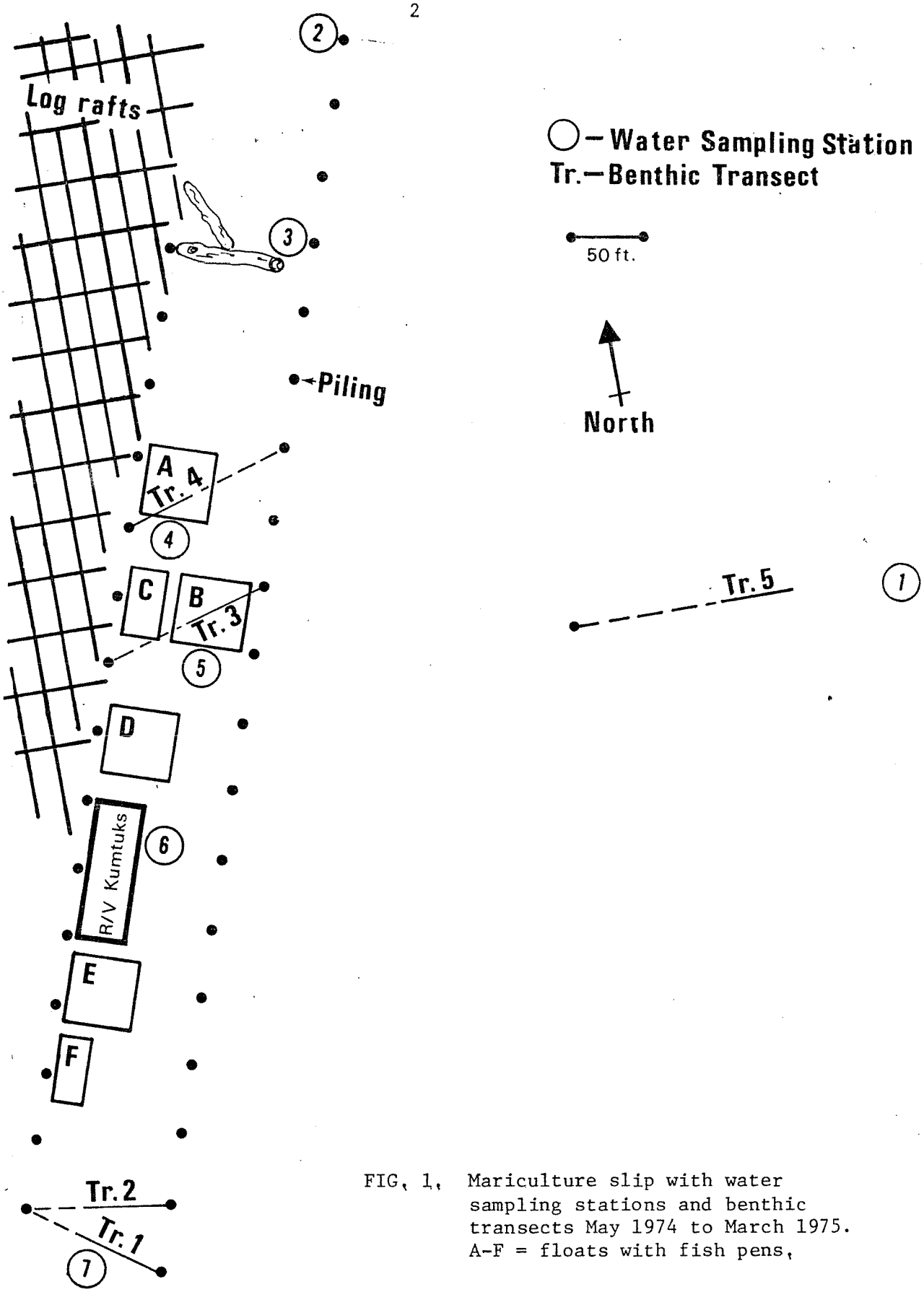


FIG. 1. Mariculture slip with water sampling stations and benthic transects May 1974 to March 1975. A-F = floats with fish pens.

of the fish were held in net pens. The majority of the Weyerhaeuser fish were held in two 50-ft x 50-ft square floats each containing four pens 20 ft x 20 ft x 11 ft deep. Float A held 36,000 fish, while the high-density Float B held 72,000 fish. Both were kept in the positions seen in Fig. 1. from May 1974 to March 1975, while the other floats were moved occasionally. All of the fish in these two floats were fed Oregon Moist Pellets (OMP).

Objectives

I. Evaluate the effect of the mariculture facility on the water quality of Henderson Inlet by measuring the temperature, salinity, chlorophyll *a*, phaeophytin, dissolved oxygen, ammonia, soluble nitrate, soluble ortho-phosphate, and total organic carbon concentrations in the water at seven stations (see Fig. 1) around the facility sampled monthly during one growing season. This phase of the investigation was essentially completed in 1974.

II. Evaluate the effect of the mariculture facility on the benthic community during the 1974 and 1975 growing seasons:

A. Under the salmon pens during a single growing season (1974 and replicated in 1975).

B. In an area which had been under salmon pens during the 1974 growing season but not during the 1975 growing season, in order to measure the recovery rate of the benthic community.

C. Under the salmon pens during both the 1974 and 1975 growing seasons

D. At control stations in 1974 and 1975

E. By measuring and statistically comparing:

1. Species composition of the benthic epifauna and infauna with special reference to indicator species
2. Species diversity of the benthic infauna as related to:
   a. sedimentation rate
   b. carbon and nitrogen composition of the sediments
   c. benthic oxygen consumption
   d. sediment particle size distribution
   e. temperature of the overyling water
   f. salinity of the overlying water

METHODS AND MATERIALS

Water Samples

Water Sampling Stations

Seven water sampling stations were positioned in and around the mariculture facility (Fig. 1). Station 1 was located halfway between the Weyerhaeuser log dump and the eastern shore of Henderson Inlet (approx 600 ft from the mariculture facility). Station 1 was assumed to be a pristine control. Station 2 was located at the northern end of the mariculture slip, 300 ft from the nearest production pen (Float A). Station 3 was located 150 ft north of Float A. Stations 4 and 5 were located in the center of Floats A and B, respectively. Station 6 was located off the eastern side of the R/V Kumtuks, 150 ft south of Float B, and in the center of the mariculture activity. Station 7 was positioned

on the south end of the mariculture slip, 500 ft from Float A, and was considered to be a control station located within the log rafting area. All water sampling stations were approx 25 to 30 ft deep at high tide and 15 to 20 ft deep at low tide.

Water sampling commenced in March 1974. The stations were sampled monthly until December 1974, when some of the pens were moved around and harvesting began. The stations located at or near the pens were not sampled until May because the final location of the pens was not determined until the fish were moved in. Sampling started 1 hr before the morning low tide at Station 1 and each station was sampled respectively until approx 2 hrs after low tide, when Station 7 was sampled. Water samples were collected from 1 m below the surface (designated by "A" after the station number) and 1 m above the bottom (designated by "B" after the station number) of each station. Temperature, salinity, and dissolved oxygen at Stations 1, 5, and 6 and chlorophyll a, phaeophytin, and ammonia at Stations 1 and 6 were measured every 3 hrs from noon September 15 to noon September 16, 1974.

The temperature, salinity, and dissolved oxygen were measured in situ with probes. Starting in August, the Winkler method (described under calibration of the dissolved oxygen meter) was used to measure dissolved oxygen. The chlorophyll a, ammonia, soluble ortho-phosphate, total phosphorus, soluble nitrate, and total organic carbon samples were collected with a 2.5-liter van Dorn bottle. After August, the dissolved oxygen samples were collected with the van Dorn bottle and placed in 300-ml-BOD bottles, then fixed with manganous sulfate and alkaline iodide. The water samples to be analyzed for chlorophyll a

and ammonia were placed in 120-ml milk dilution bottles. The water samples to be analyzed for soluble ortho-phosphate, total phosphorus, and soluble nitrate were placed in 1-liter French square bottles with 40 mg/l mercuric chloride added as a preservative. The samples to be analyzed for total organic carbon were placed in 1-liter French square bottles with 5 ml of concentrated sulfuric acid added as a preservative.

Analysis of Water Samples

Temperature and Salinity. Measurements were made with a Kahlsico Model RS5-3 temperature and salinity probe. The instrument was calibrated the day before each monthly sampling date, using the standard resistance technique described by the manufacturer. The instrument measurement error is $\pm$ 0.05 ppt for the salinity probe.

Dissolved Oxygen. Measurements were made with a YSI Model 51A dissolved oxygen meter. The meter was calibrated the day before each sampling date, using the azide modification of the Winkler method as outlined by the American Public Health Association (APHA) et al (1971, pp. 447-481), except that an equivalent concentration of phenylarsine oxide was substituted for the sodium thiosulfate titrant. The meter has a cumulative measurement error of $\pm$ 0.4 ppm.

Chlorophyll a. Measurements were made with a Turner fluorometer Modell 111 as outlined by Strickland and Parsons (1972, pp. 201-203) except the chlorophyll a was extracted as follows: 100 ml of sample was filtered through a 2.4-cm Whatman GF/C glass fiber filter as soon

as possible on the R/V Kumtuks. The sample filters were stored for up to 24 hrs in a dessicator in a dark freezer, then ground in a tissue grinder with 90% acetone and filtered through another GF/C filter. The extract was made up to 10 ml with more 90% acetone, thoroughly mixed, then read in the fluorometer against a 90% acetone blank. The extract was acidified with one drop of 1 N hydrochloric acid to convert the chlorophyll to phaeophytin and read in the fluorometer again. The chlorophyll a concentration was calculated using a conversion factor derived from analysis of water samples collected from Henderson Inlet during a plankton bloom using the spectrophotometric technique outlined in Strickland and Parsons (1972, pp. 193-194) except for the extraction process described above. A precision of $\pm$ 8% of the value expressed in µg chlorophyll a/l is attained with this method.

Ammonia. The method of Strickland and Parsons (1972, pp. 87-89) was followed but the amount of sample and reagents was reduced by one-fifth. The optical density of the sample was measured with a Gilford Model 300-N microsample spectrophotometer. Two replicates were analyzed from each sample. All samples were filtered through a 2.4-cm GF/C glass fiber filter and subsequently analyzed within 6 hr of collection. The method has a precision of $\pm$ 10% of the value expressed as µg ammonia/l.

Nitrate plus Nitrite. Measurements were made with a Technicon Automated Analyzer Two at the Weyerhaeuser water quality laboratory in Longview, Washington. The exact methods are very complicated and

are currently being written, so only an outline of the Technicon methods will be given (this also applies to the ortho-phosphate, and total phosphorus analyses). The nitrates were reduced to nitrites in a cadmium column. Sulfanilamide was added to form a diazo compound which was then coupled in an acid solution with N-1 naphthyl-ethylenediamine dihydrochloride to form an azo dye. The optical density of the azo dye was then measured spectrophotometrically. Baseline and calibration adjustments were made using an artificial seawater solution containing 25 g/l sodium chloride and 3.9 g/l magnesium sulfate (a salinity of 28.9 ppt). Values are reported to the nearest µg nitrate/l.

Ortho-phosphate. Measurements were made with a Technicon AA2. A portion of the sample was centrifuged, then reacted with ammonium molybdate and potassium antimonyl tartrate in an acid medium to form an antimony-phospho-molybdate complex. The optical density of this stable complex was measured spectrophotometrically after the addition of ascorbic acid. Baseline and calibration adjustments were made using the above mentioned artificial seawater solution. Values are reported to the nearest µg phosphate/l.

Total Phosphorus. Measurements were made with the Technicon AA2. Organic phosphorus was converted to ortho-phosphate by a manual digestion using ammonium persulfate. The phosphate was then measured using the technique described for orthophosphate. Values are reported to the nearest µg phosphorus/l.

Total Organic Carbon. Measurements were made with an Oceanography Instruments total organic carbon analyzer at the Weyerhaeuser Harbor Island laboratory. Ten ml of sample were placed in a conditioned 10-ml ampule. The sample was acidified with 0.25 ml of 20% sulfuric acid and purged with nitrogen for 6 min, then sealed into the ampule. The samples were digested for 4 hrs in a pressure cooker and allowed to cool. The ampules were broken in the ampule analyzing unit, along with four to six reagent blanks and several sucrose standards. The peak areas on the disc integrator or electronic integrator were then measured. The measured areas were corrected with the average blank value and calibrated with the standard curve of counts vs. ppm carbon to calculate mg carbon/kg.

Benthic Sampling

Benthic Transects

Five underwater transects were placed at the mariculture and control sites (Fig. 1). Four of the transects consisted of a length of 1/4-inch thick polypropylene line stretched across the width of the mariculture slip (approx 100 ft) and tied to a piling at each end, 1 ft above the bottom. One end of each transect was marked at 2-ft intervals, starting several feet from the piling and continuing for 50 ft toward the other piling. This 50-ft section was the functional part of the transect. Transects 1 and 2 were located on the south end of the mariculture slip (Fig. 1) with the functional part of them on the east side of the slip. These were used as control transects, with sediment characteristics typical of the slip area, while located 400 ft from the nearest production float (Float B). Preliminary studies

indicated that the bottom sediments (amount of bark) and general faunal assemblage at Transects 1 and 2 were similar to that under the pens. Transects 3 and 4 were located under Floats B and A, respectively. Transect 5 consisted of a length of 1/4-inch diameter polypropylene line stretched 150 ft eastward from the base of the outer dolphin (Fig. 1) to an anchor. The functional 50-ft section (marked at 2-ft intervals) was located on the eastern end of the transect (near the anchor), 350 ft from Float B. Transect 5 was considered to be a pristine benthic control area. Preliminary studies indicated that bottom sediments at Transect 5 consist mostly of fine silt with very little bark, which is typical of the sediments at Henderson Inlet outside of the log-rafting area.

Benthic Communities

Benthic Epifauna. The benthic epifauna was enumerated monthly by SCUBA divers along the 50-ft section of the transect. All visible macrofauna within 3 ft of the transect was recorded on a waterproof slate. Therefore, an area of 150 ft^2 was sampled at each transect. The organisms were identified to genus and species (if possible) so that changes in abundance and species composition of the epifauna can be documented.

Benthic Infauna. Five random core samples were collected at each transect by SCUBA divers in April, June, and August, and at Transects 1, 3, and 5 in December. The 25 positions marked along a transect were assigned their respective numbers starting with 1 at the mark nearest the piling or anchor and ending with 25 near the middle of the transect.

The series of 25 positions was then randomized. Since five different positions were to be sampled with non-replacement at five different points in time, five of each of the numbers from 1 to 5 were randomized in a series. This random series of 25 numbers was then matched with the random series of 25 positions on the transect and the process repeated for each transect separately. To provide additional spacing of samples along the transect, the even- and odd-numbered samples were collected 3 ft left and 3 ft right (respectively) of the position on the transect line, so all samples were at least 7 ft apart. This spacing was necessary so that stirring up of sediments while collecting one sample did not disturb the other sample positions along the transect. While SCUBA diving along the transect for any reason, conscious effort was always made to refrain from stirring up the sediments. Buoyancy vests were used to maintain a constant distance above the transect.

SCUBA divers collected the infauna samples with a cylindrical corer measuring 15.5 cm inside diameter and 17.5 cm in length. The corer sampled an area of 0.019 m^2 and collected 0.0027 m^3 of sediment, while the five samples collected at each transect sampled a total area of 0.095 m^2 and a total volume of 0.0135 m^3. The core samples were screened through a 0.42-mm mesh screen within several hours of collection. The screened samples were stored up to 2 months in 10% buffered Formalin with 0.1 g/l rose bengal stain (Mason and Yevich, 1967). The fixed and stained samples were then washed in a 0.40-mm screen and put into a white porcelain tray, where the organisms were sorted from the debris. They were roughly sorted to species and stored in 70% ethyl alcohol with 10% glycerine for later identification and verification.

A species abundance list was constructed and two diversity indices were calculated (Fager, 1972)-- the unscaled Shannon-Weaver diversity index which measures both the richness and evenness components:

$$H' = -\sum_{i=1}^{s} \frac{n_i}{N} \ln \frac{n_i}{N}$$

and Gleason's diversity index which measures only the richness component:

$$d = (s - 1)/\ln N$$

where: N = total number of individuals

s = total number of species

n_i = number of individuals of the i^{th} species

Sediment Sampling

SCUBA divers collected five random core samples at each transect in April, June, and August and at Transects 1, 3, and 5 in December, immediately before collection of the infauna cores at the same positions along the transects except 2 ft to the right at even-numbered positions and 2 ft to the left at odd-numbered positions, so the core samples were at least 6 ft apart along the transect. These sediment corers measured 2.8 cm in inside diameter and 3.0 cm in length. Within 1 hr of collection the corer and sample were frozen. The 15.7-cc frozen sample was removed from the corer and stored frozen in a "Whirl-Pac" bag until analysis at the Weyerhaeuser research laboratory at Harbor Island, Seattle.

At the laboratory, the cores were placed into a 1200-ml freeze-dry flask with a small amount of distilled water (approx 10 ml) which was used to rinse out the "Whirl-Pac" bag. The contents of the flask was

thawed (approx 15 to 20 min) and then freeze-dried. The dry powder was homogenized with a mortar and pestle, then stored in a tightly-covered jar until being analyzed for carbon and nitrogen. The first two sets of cores (from April and June) were analyzed for carbon with an AMICO C-H analyzer and nitrogen with a Kjeldahl digestion technique. The remaining cores were analyzed for carbon and nitrogen with a Carlo Erba C-N-H-O analyzer.

RESULTS

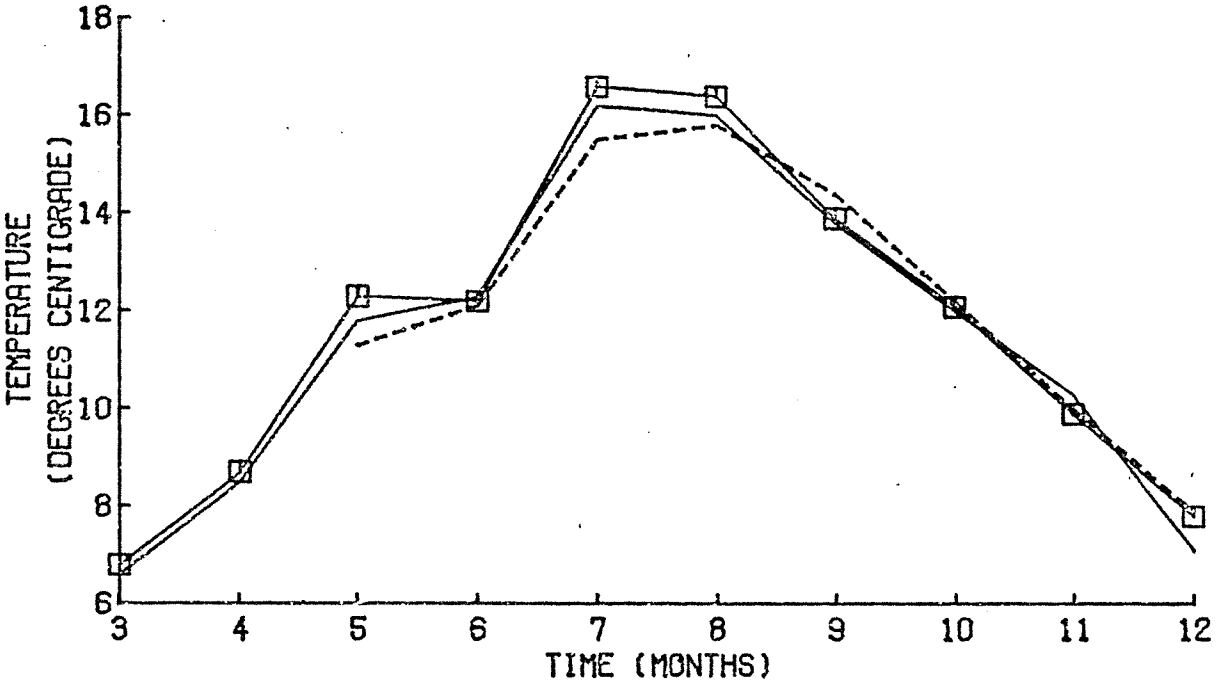
Water Quality

Monthly plots of all water quality parameters collected at the surface and bottom of Stations 1, 3, and 5 were constructed so that the pattern of seasonal variation in these parameters at the two control stations and the high-density pens could be visualized. In order to visualize the variation of the water quality parameters between stations, each parameter was plotted at all stations on the month when the variation of the parameter between stations was greatest. The results of the 24-hr water sampling experiment are plotted along with tidal height to demonstrate the diurnal and tidal effects on temperature, salinity, chlorophyll *a*, phaeophytin, DO, and ammonia.

Temperature and Salinity

The temperature and salinity regime at Henderson Inlet was typical of a shallow bay in Puget Sound, generally low in the winter and high in the summer. Surface temperatures exhibited one small peak in May and June with the highest peak (16.6) in July and August (Fig. 2A), while

A. (LINE=STATION 1A; DASH=STATION 5A; SQUARE=STATION 7A)



B. (LINE=STATION 1B; DASH=STATION 5B; SQUARE=STATION 7B)

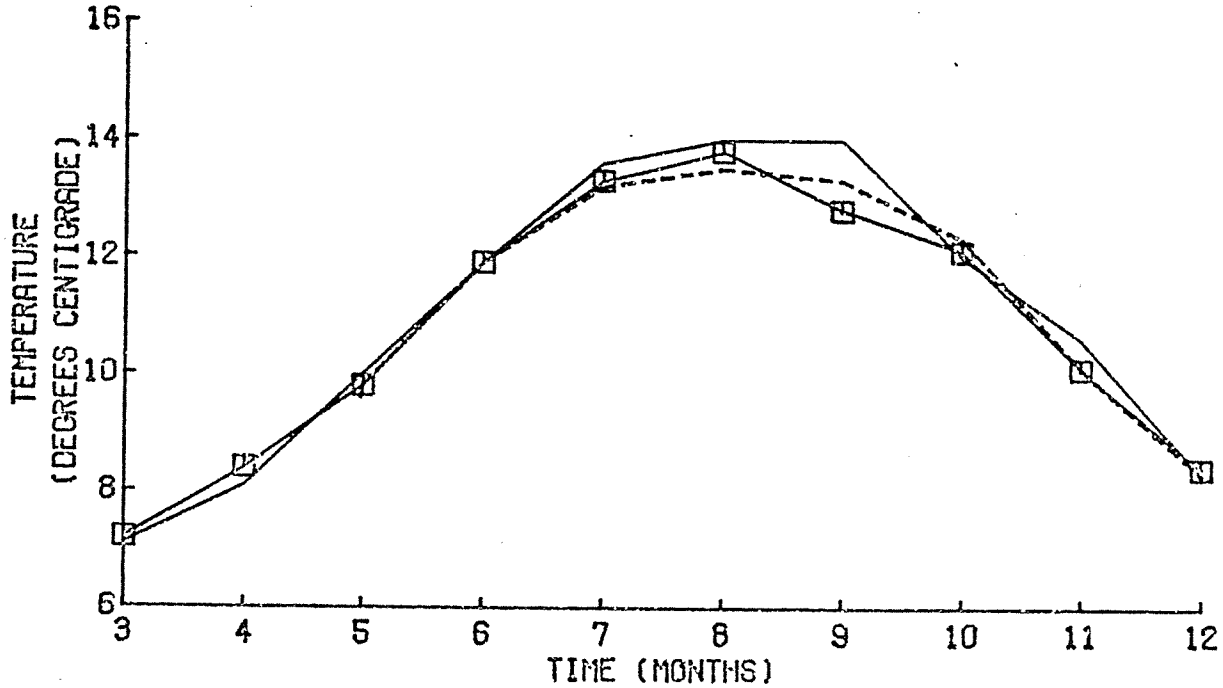


FIG. 2. WATER TEMPERATURE AT STATIONS 1, 5, AND 7 AT HENDERSON INLET MARCH TO DECEMBER 1974.

temperatures near the bottom exhibited one peak July through September (Fig. 2B). There was very little variation in temperature and salinity between stations. The surface temperature was obviously affected by the diurnal fluctuation in air temperature during the summer, as indicated in Fig. 3. The water temperature peaked during the afternoon slack tide and decreased to its minimum during the evening slack tide. The temperature near the bottom was not noticeably affected by the air temperature.

The salinity at the surface and bottom peaked at the end of each of the seasonal temperature peaks (Fig. 4). The salinity near the surface peaked on the outgoing tide (Fig. 5). The salinity near the bottom remained relatively constant through the tidal cycle.

Phytoplankton

Chlorophyll a and phaeophytin concentrations are directly related to phytoplankton density, since all algal cells contain some chlorophyll a and chlorophyll a readily degrades to phaeophytin. As indicated by chlorophyll a concentration (Fig. 6), there were three major phytoplankton blooms near the surface in April, July, and September and two major blooms near the bottom in July and September. They were generally denser in the surface waters within the mariculture slip than at the outer control station (Figs. 6A and 7A). The phytoplankton density near the bottom did not differ much between stations, except during the bloom in July when the density near the bottom was much higher at the outer control station than at any stations within the mariculture slip. The phytoplankton density at the surface and bottom fluctuated both

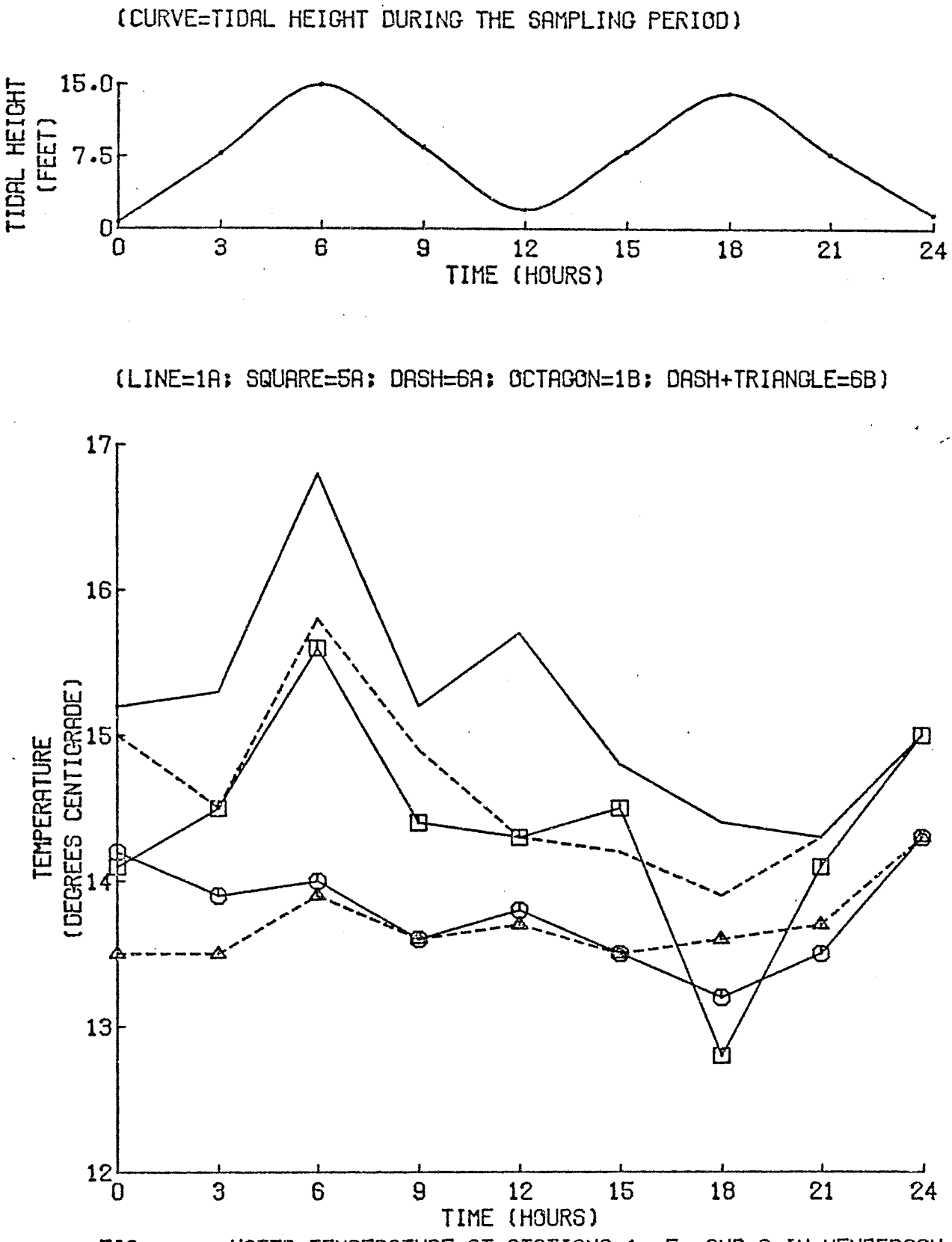
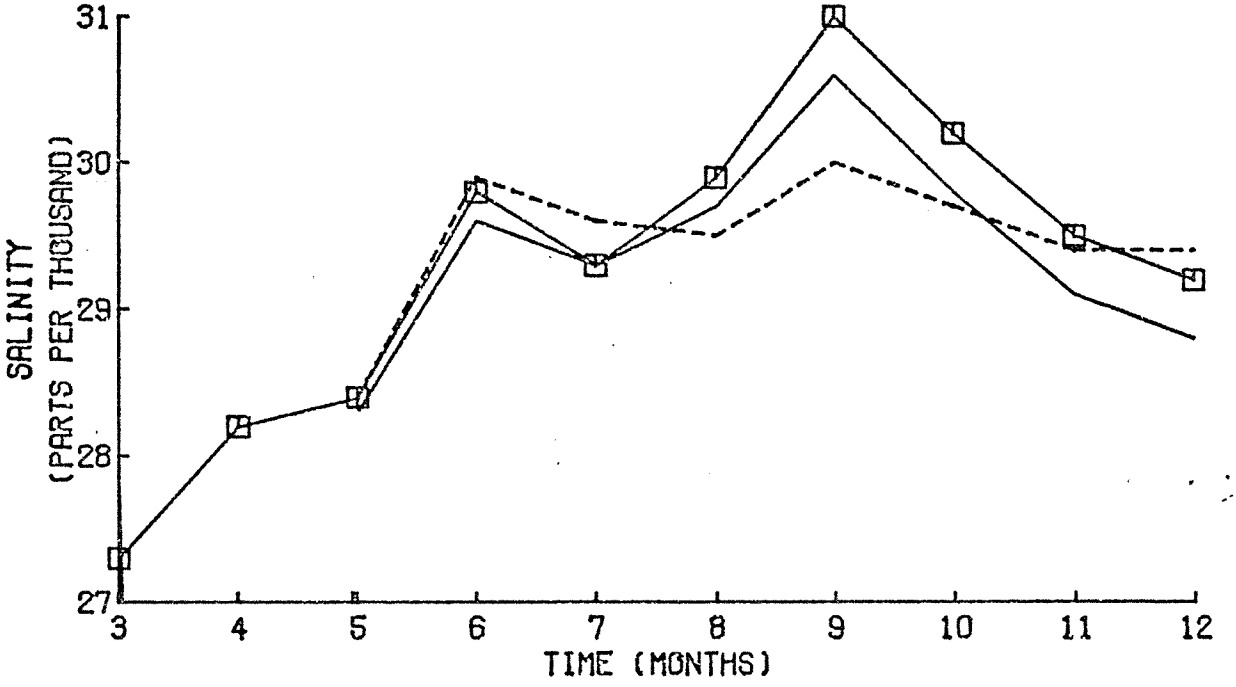


FIG. 3 . WATER TEMPERATURE AT STATIONS 1, 5, AND 6 IN HENDERSON INLET FROM NOON SEPT. 15 TO NOON SEPT. 16, 1974.

A. (LINE=STATION 1A; DASH=STATION 5A; SQUARE=STATION 7A)



B. (LINE=STATION 1B; DASH=STATION 5B; SQUARE=STATION 7B)

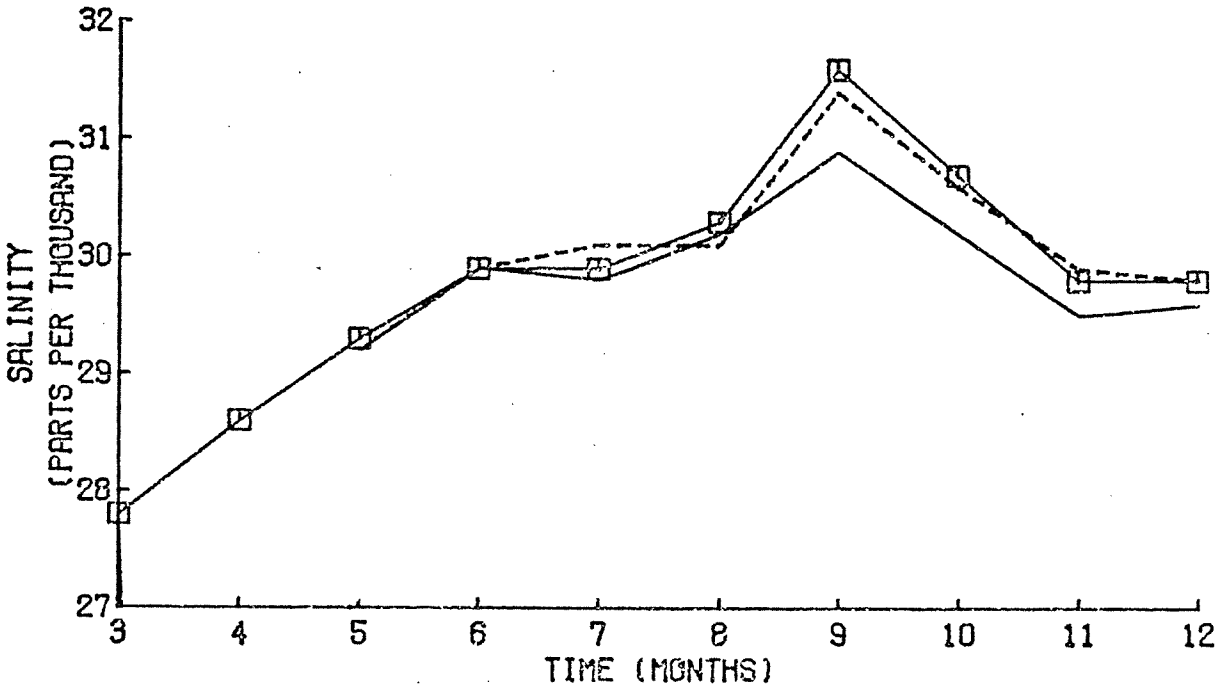


FIG. 4 . SALINITY AT STATIONS 1, 5, AND 7 AT HENDERSON INLET MARCH TO DECEMBER 1974.

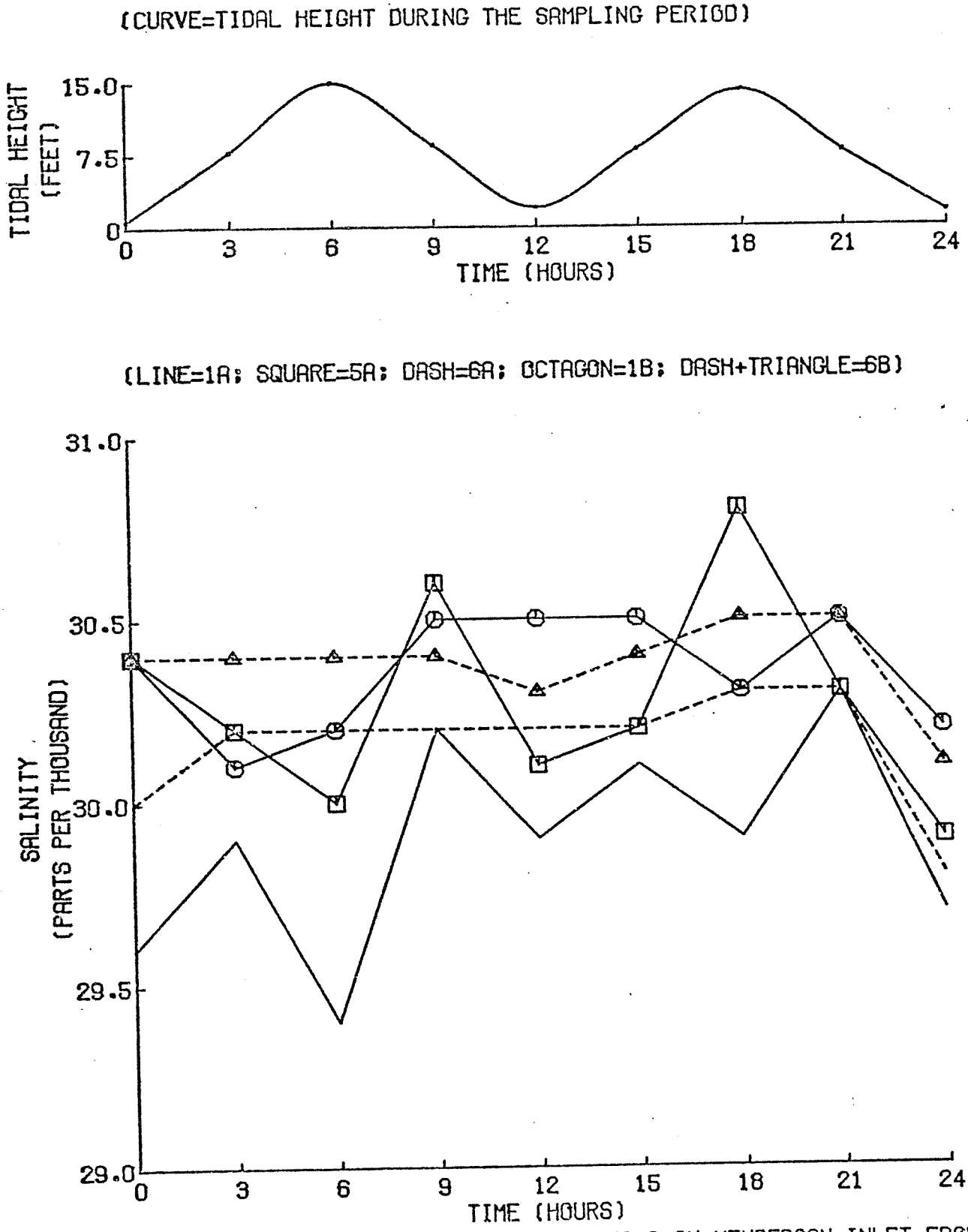
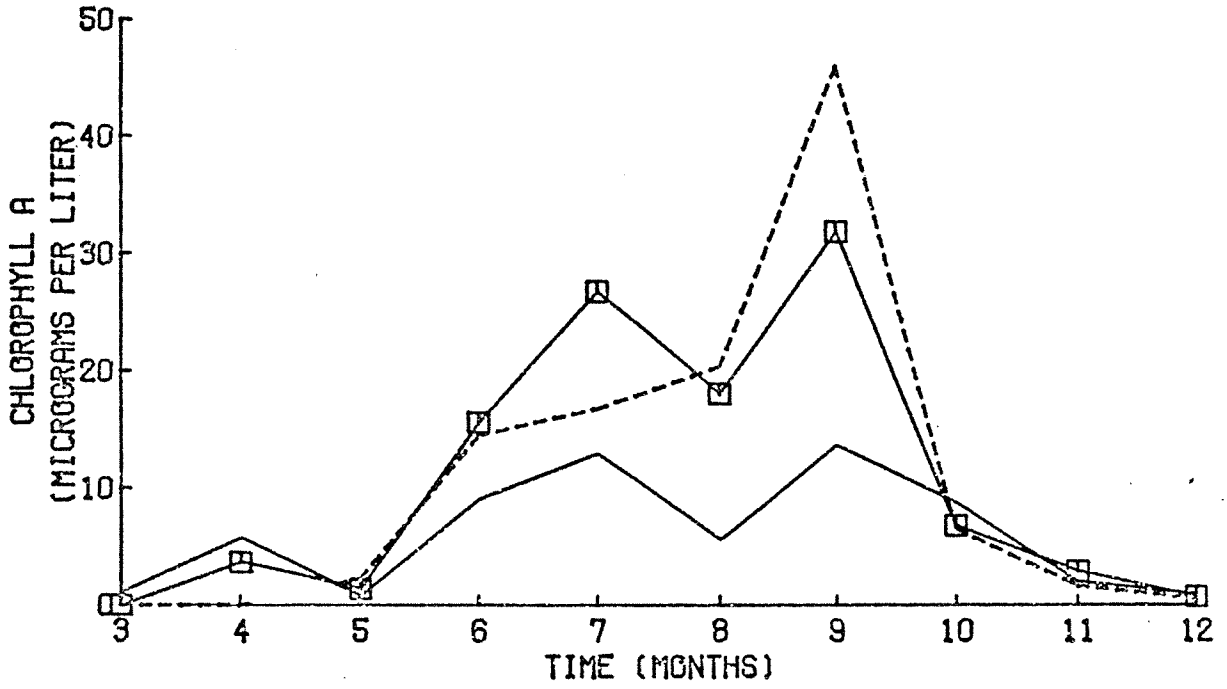


FIG. 5. SALINTY AT STATIONS 1, 5, AND 6 IN HENDERSON INLET FROM NOON SEPT. 15 TO NOON SEPT. 16, 1974.

A. (LINE=STATION 1A; DASH=STATION 5A; SQUARE=STATION 7A)



B. (LINE=STATION 1B; DASH=STATION 5B; SQUARE=STATION 7B)

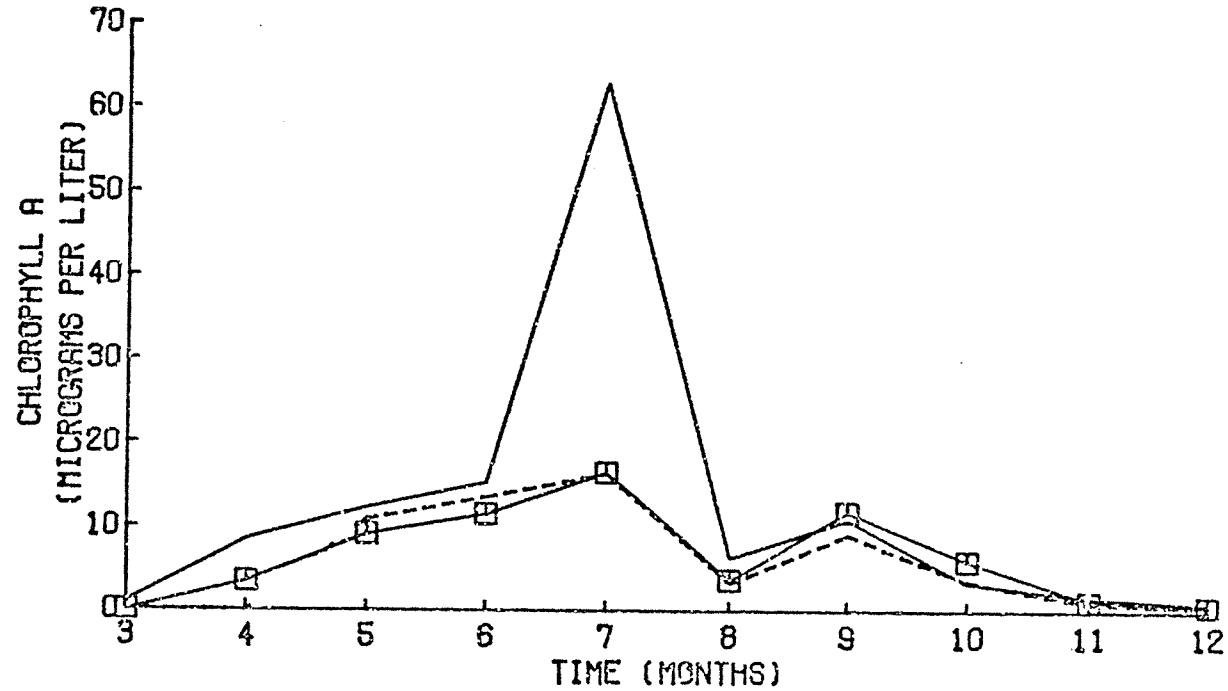
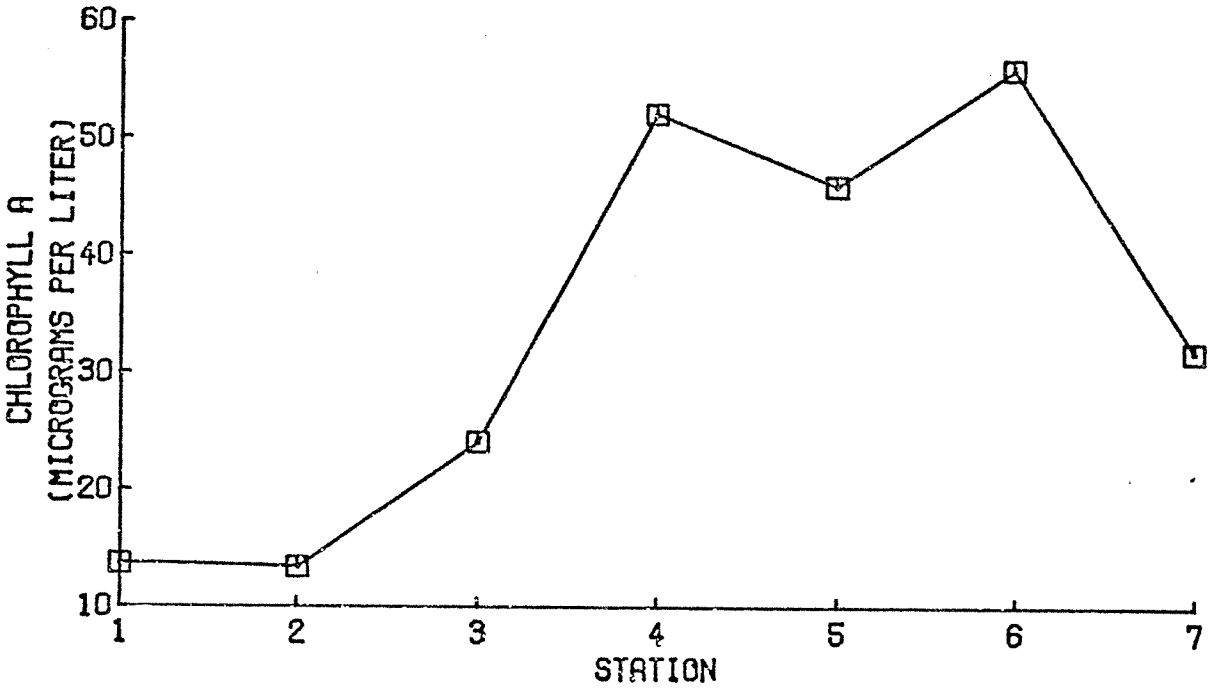


FIG. 6. CHLOROPHYLL A CONCENTRATION AT HENDERSON INLET MARCH TO DECEMBER 1974.

A. 1M BELOW SURFACE IN SEPTEMBER



B. 1M ABOVE BOTTOM IN JULY

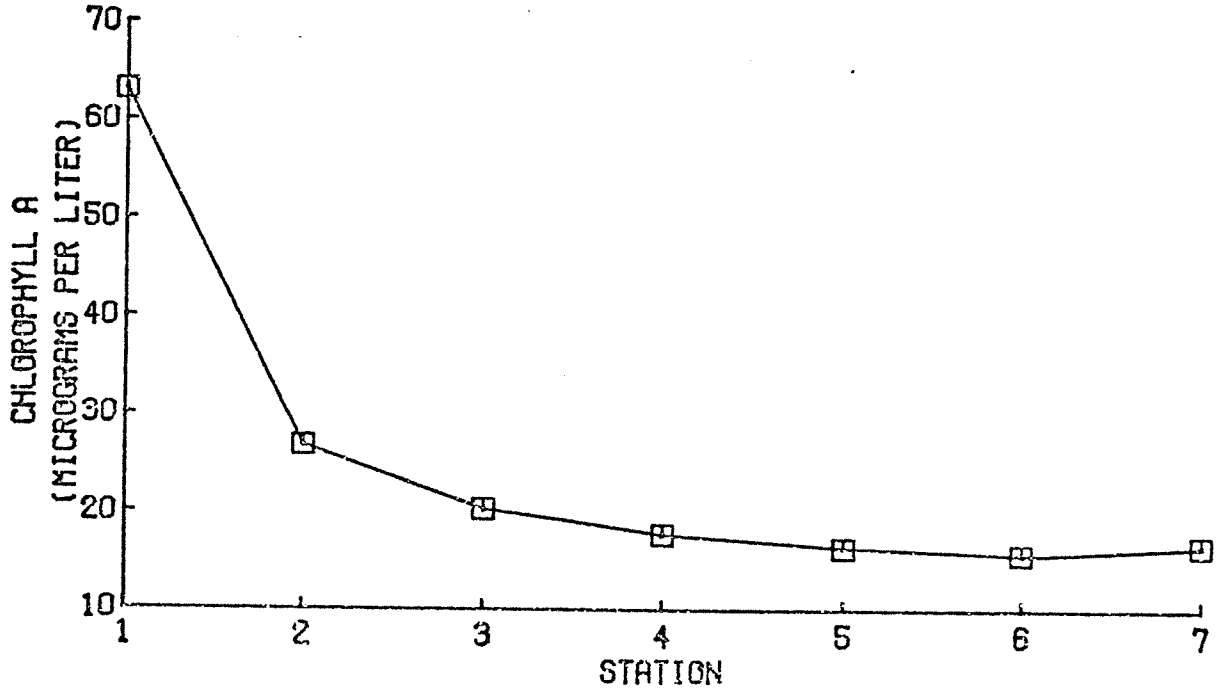


FIG. 7. CHLOROPHYLL A CONCENTRATION AT STATION 1 THROUGH 7 IN 1974 MONTH OF GREATEST RANGE.

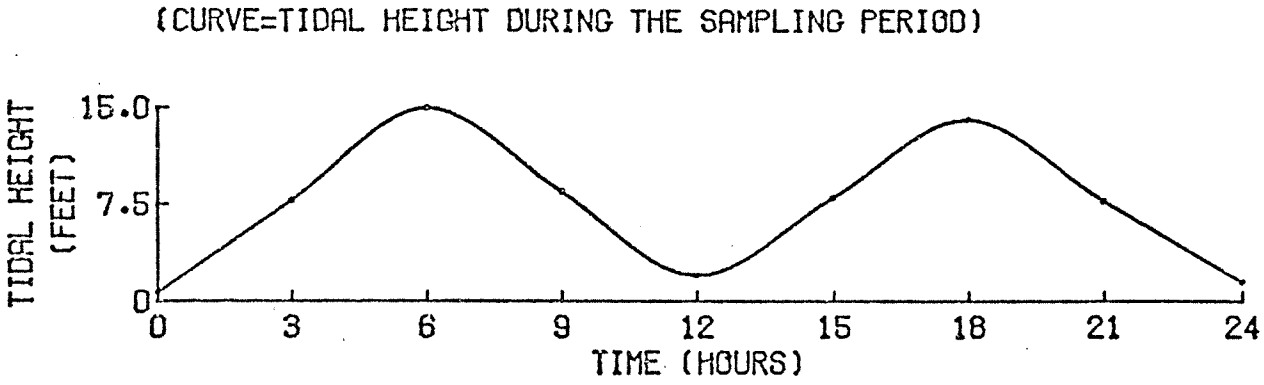
tidally and diurnally during the summer (Fig. 8), with the maximum phytoplankton density occurring during the daytime high tide and the minimum during the evening low tide.

The phaeophytin concentration in the surface waters of all stations also indicated that there were three major phytoplankton blooms (Fig. 9). The general distribution of phaeophytin between stations was similar to that of chlorophyll a (Fig. 10). Short-term fluctuations in phaeophytin concentrations were primarily caused by the tide during the summer, with peak concentrations near low tide and low concentrations near high tide (Fig. 11). Tidal fluctuations in phaeophytin were the reverse of the tidal fluctuations in chlorophyll a.

Dissolved Oxygen

Three peaks in dissolved oxygen (DO) were observed near the surface at the outer control station and near the bottom at all stations (Fig. 12), corresponding with the three peaks in phytoplankton density. The DO near the surface within the mariculture slip declined drastically in July, whereas the DO didn't drop at the outer control until August. The highest DO (13 ppm) occurred in July near the bottom at Station 3, and the lowest DO (5.6 ppm) occurred near the surface at Station 6. The general trend was toward lower DO's inside the mariculture slip than outside (Fig. 13). The greatest variation in DO between stations was found during the summer, with similar DO's at all stations in winter.

The DO near the surface and bottom appears to be affected by both diurnal and tidal factors during summer (Fig. 14), with the maximum DO occurring during the daytime high tide (except at Station 5) and the



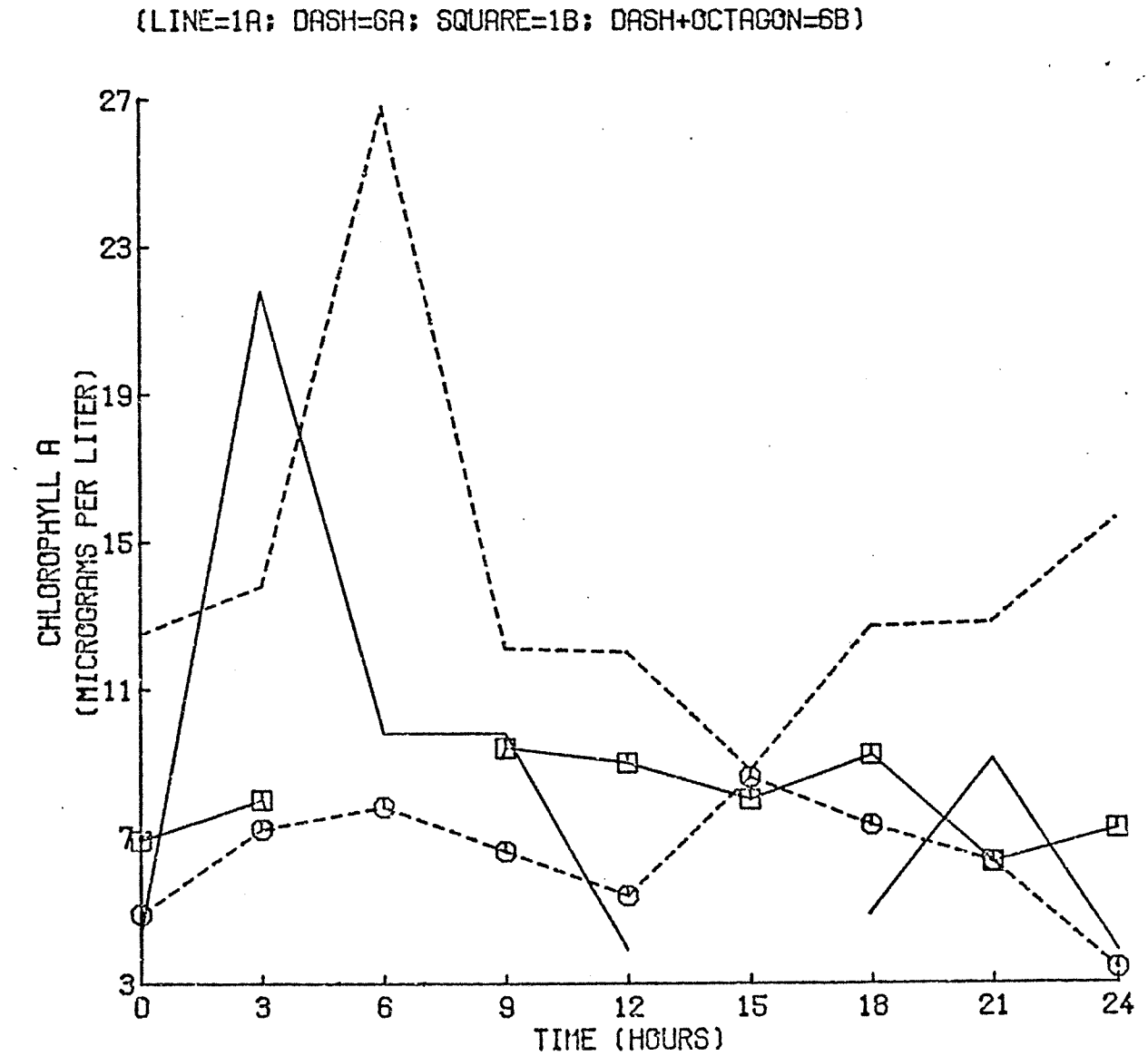
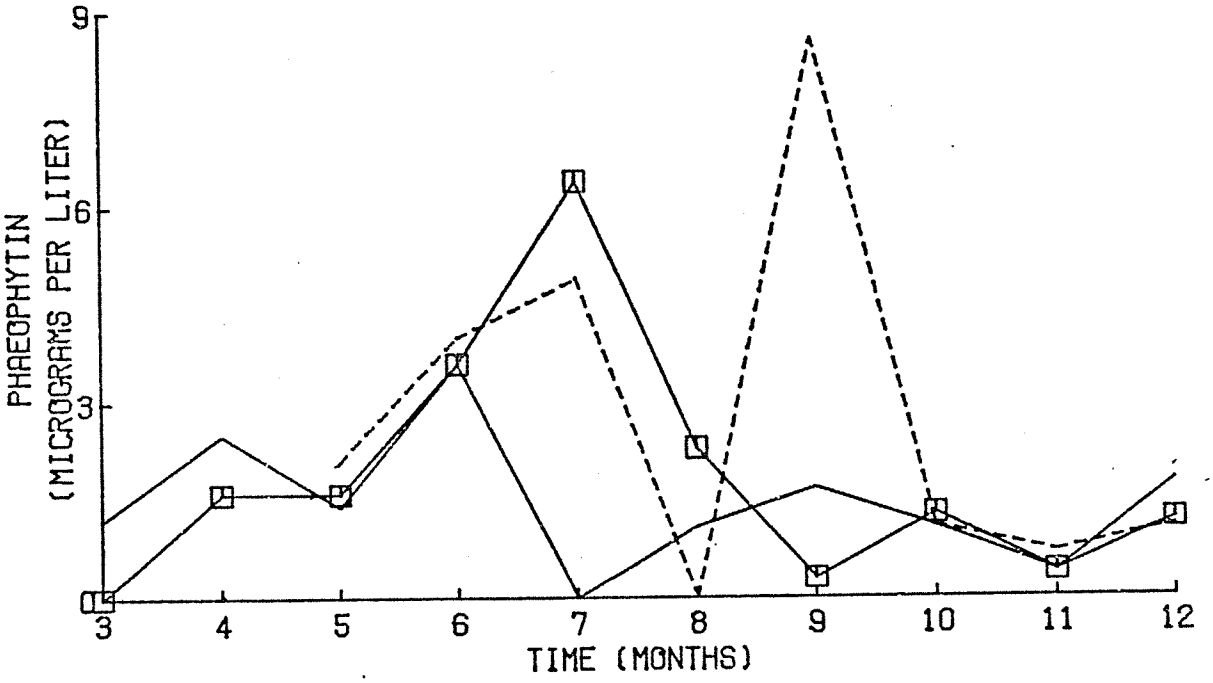


FIG. 8 . CHLOROPHYLL A CONC. AT STATIONS 1 AND 6 IN HENDERSON INLET FROM NOON SEPT. 15 TO NOON SEPT. 16, 1974.

A. (LINE=STATION 1A; DASH=STATION 5A; SQUARE=STATION 7A)



B. (LINE=STATION 1B; DASH=STATION 5B; SQUARE=STATION 7B)

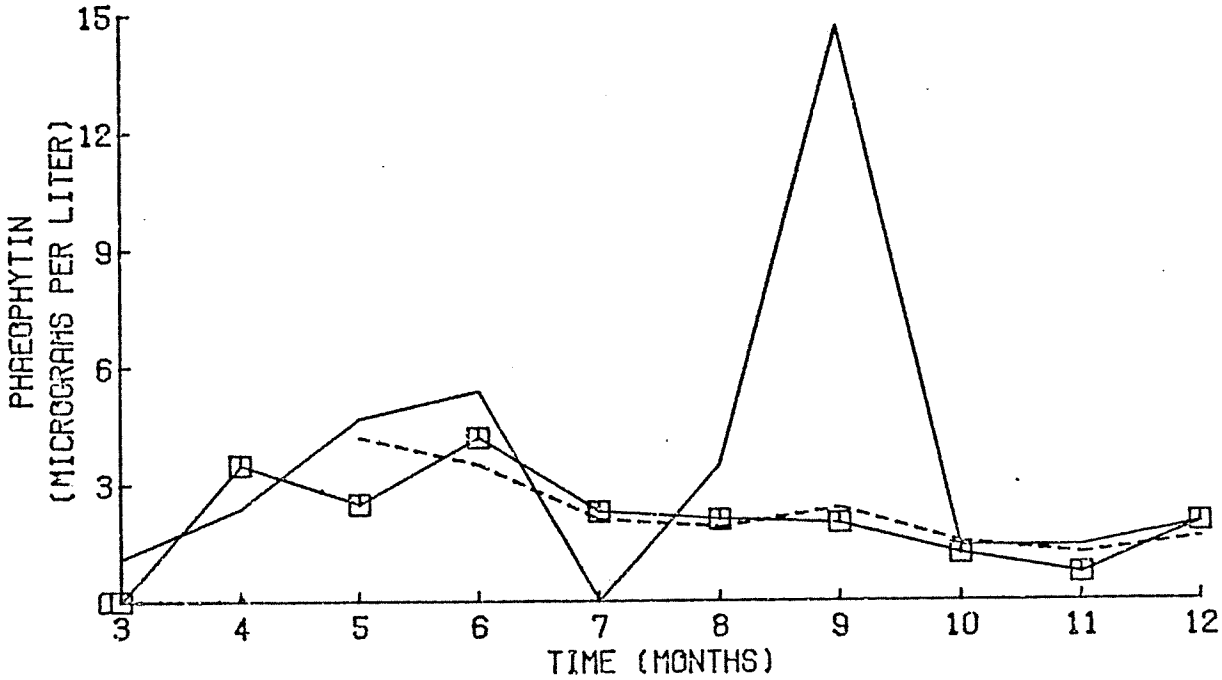
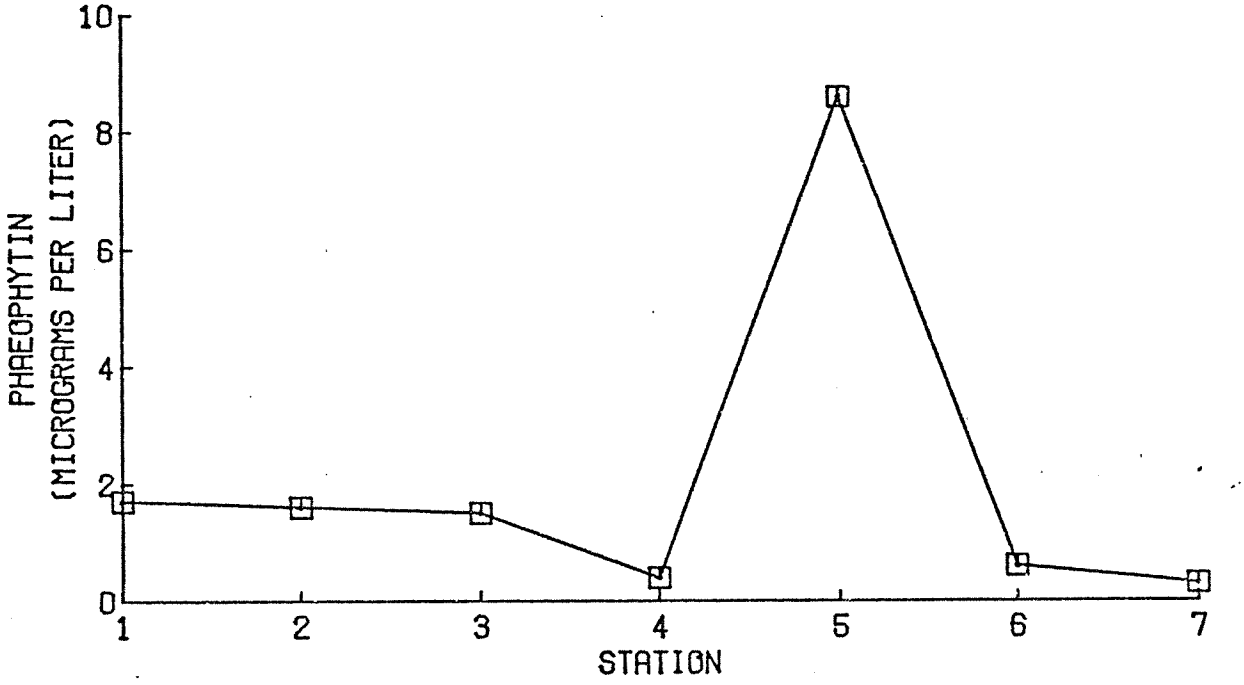


FIG. 9 . PHAEOPHYTIN CONCENTRATION AT STATIONS 1, 5, AND 7 AT HENDERSON INLET MARCH TO DECEMBER 1974.

A. 1M BELOW SURFACE IN SEPTEMBER



B. 1M ABOVE BOTTOM IN SEPTEMBER

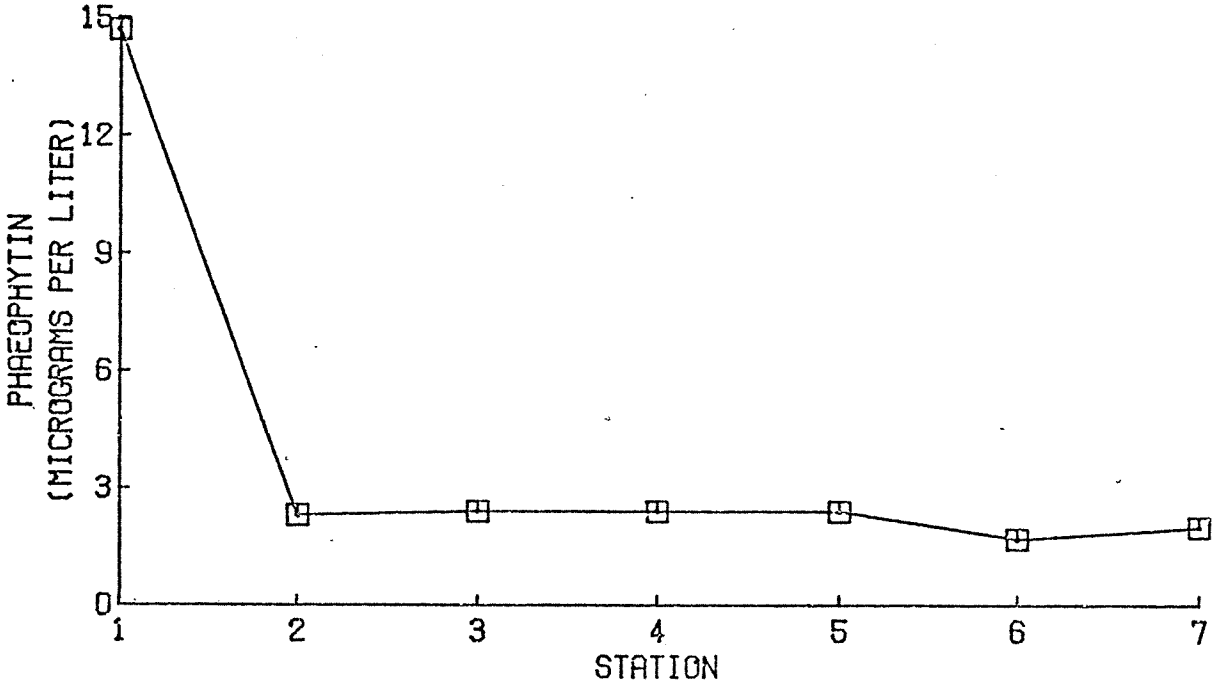
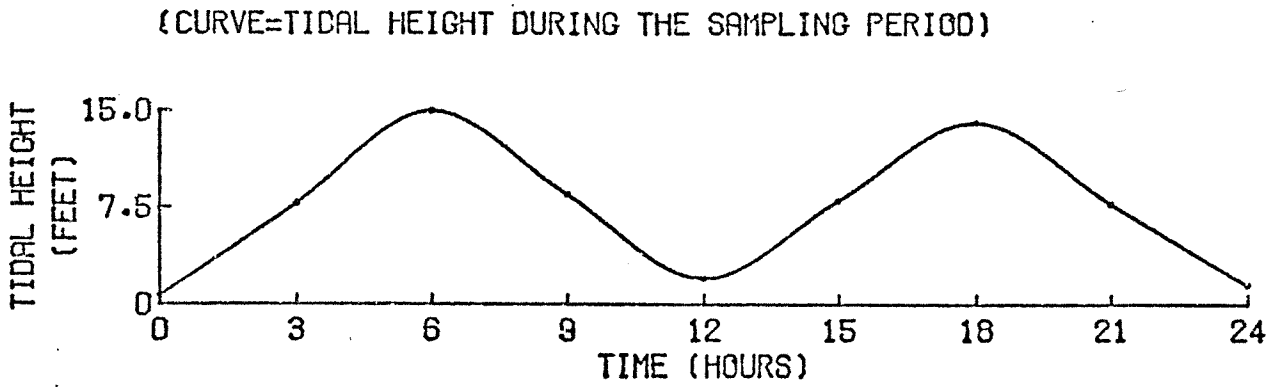


FIG. 10. PHAEOPHYTIN CONCENTRATION AT STATIONS 1 THROUGH 7 IN 1974 MONTH OF GREATEST RANGE.



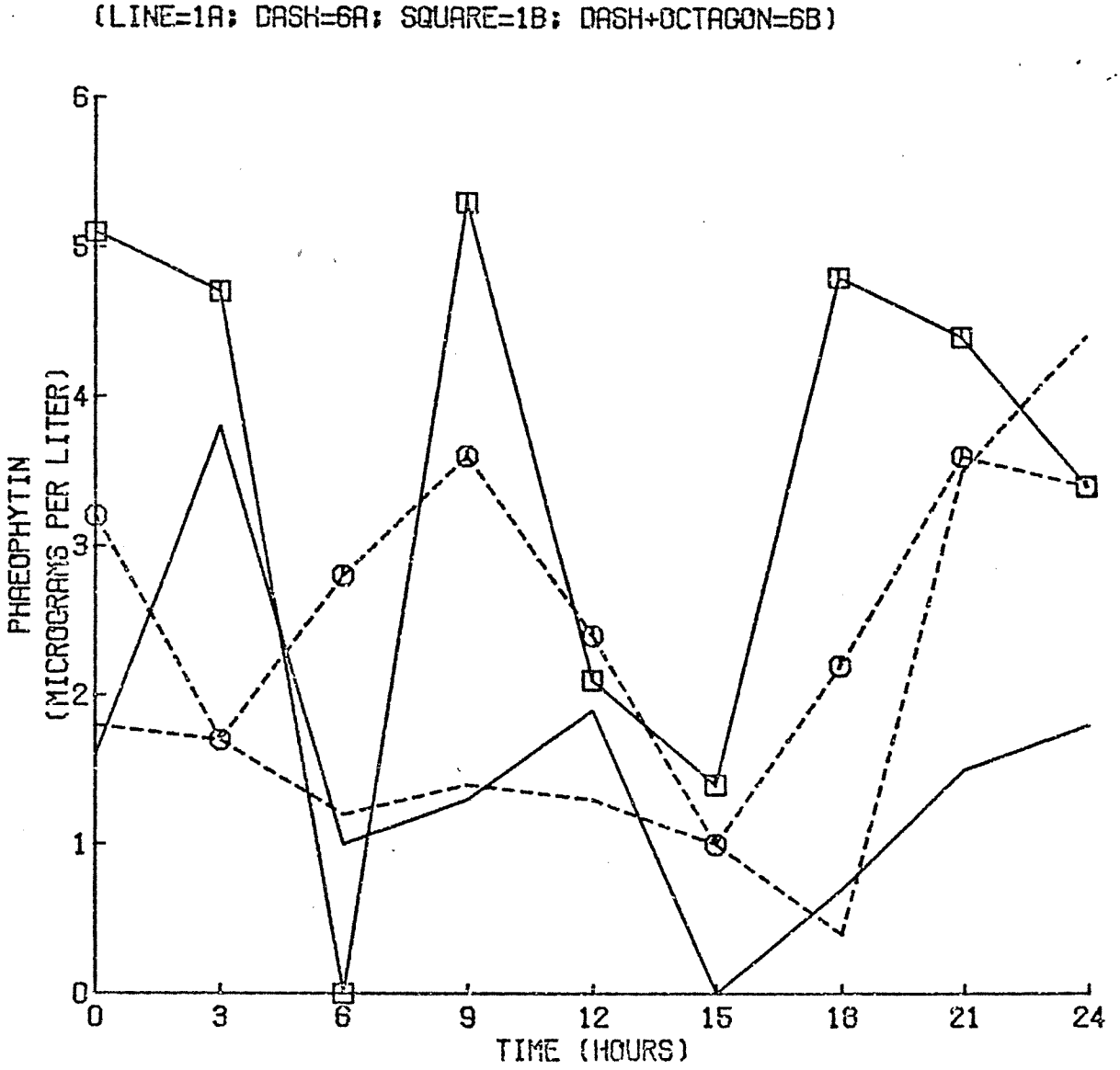
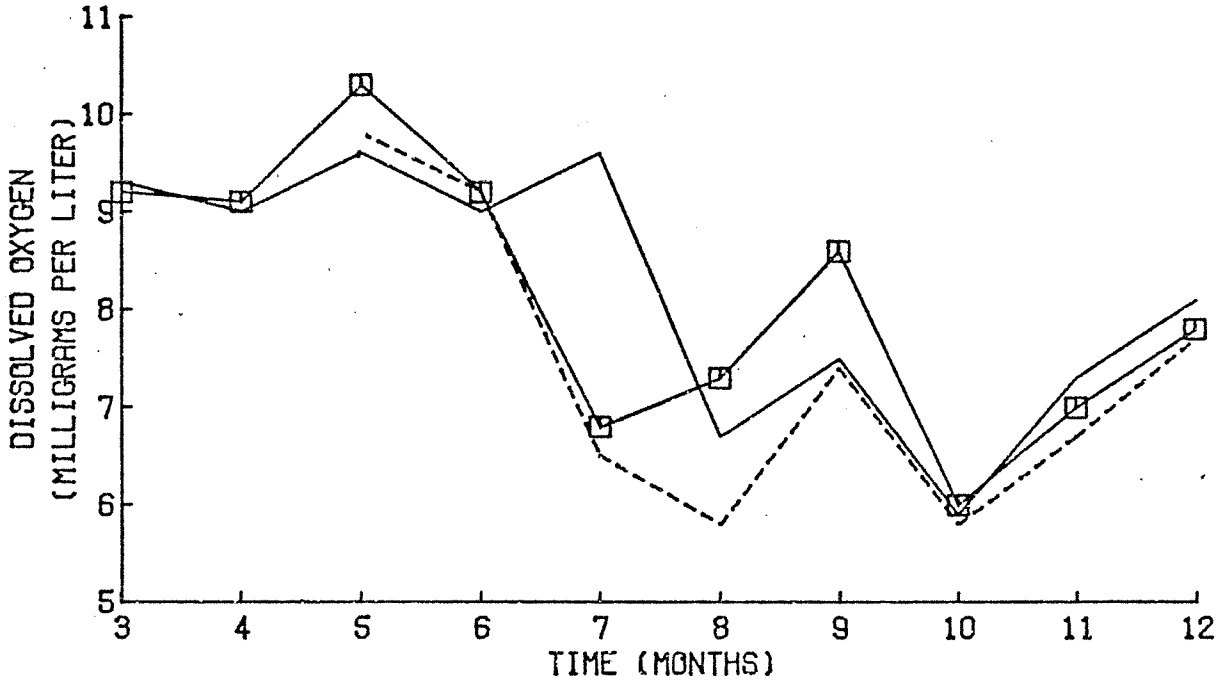


FIG. 11. PHAEOPHYTIN CONCENTRATION AT STATIONS 1 AND 6 IN HENDERSON INLET FROM NOON SEPT. 15 TO NOON SEPT. 16, 1974.

A. (LINE=STATION 1A; DASH=STATION 5A; SQUARE=STATION 7A)



B. (LINE=STATION 1B; DASH=STATION 5B; SQUARE=STATION 7B)

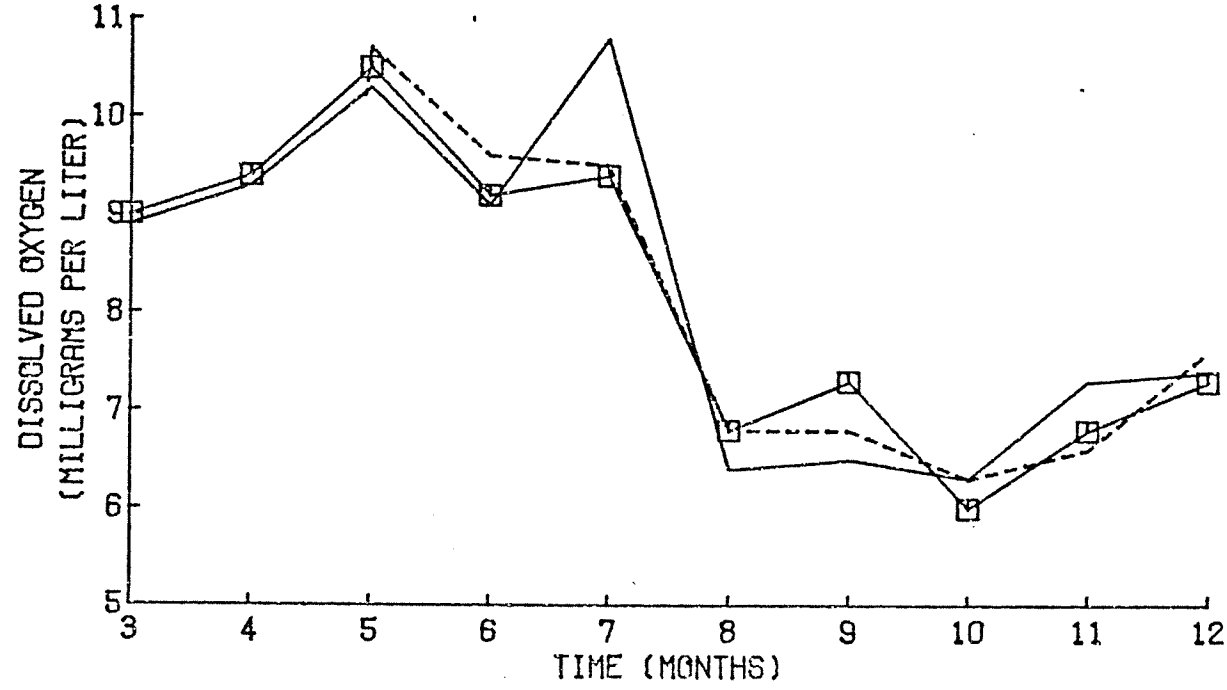
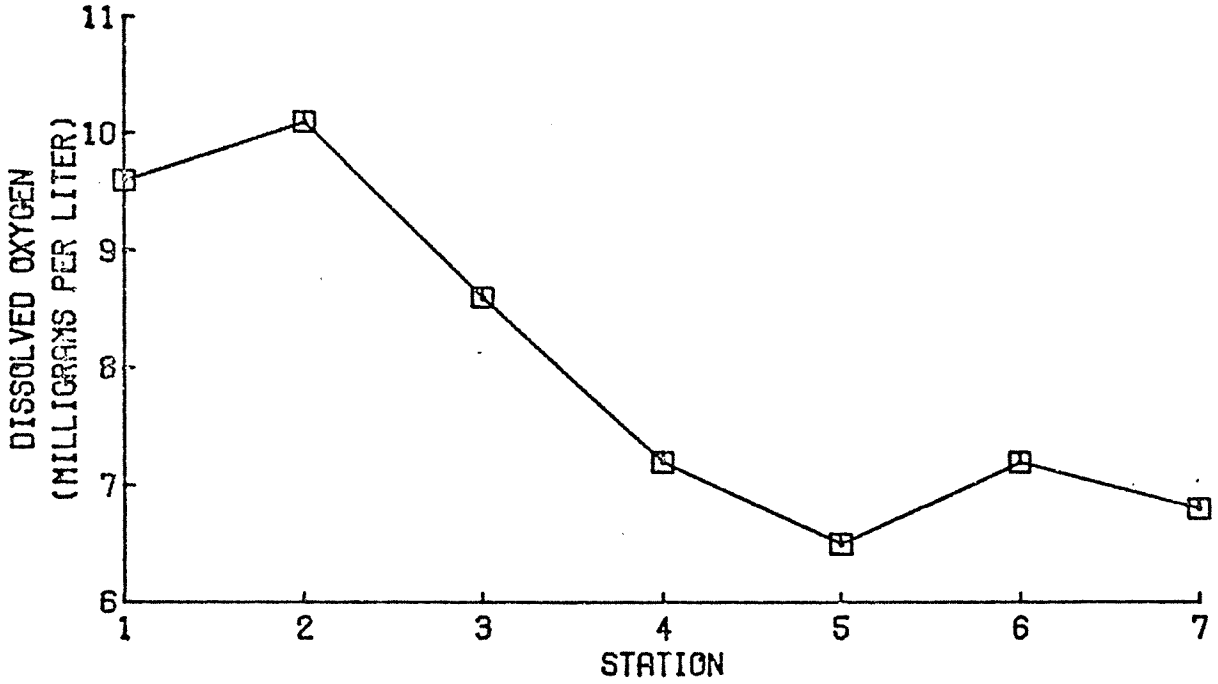


FIG. 12. DISSOLVED OXYGEN CONCENTRATION AT STATIONS 1, 5, AND 7 AT HENDERSON INLET MARCH TO DECEMBER 1974.

A. 1M BELOW SURFACE IN JULY



B. 1M ABOVE BOTTOM IN JULY

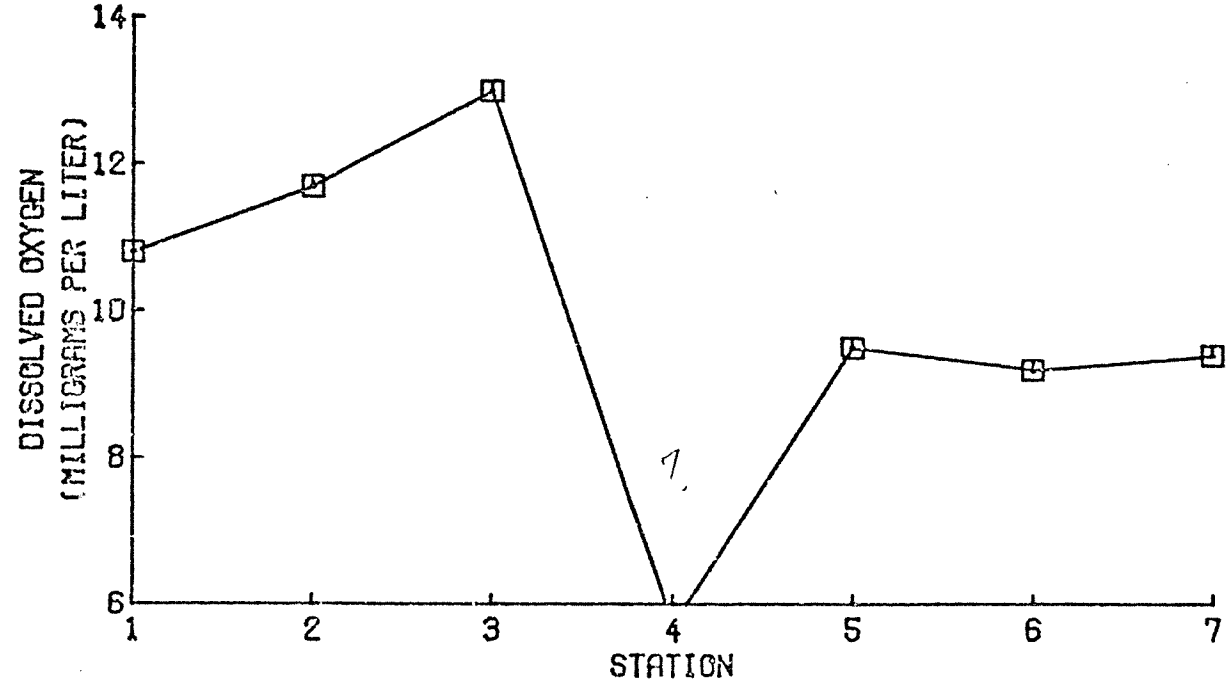
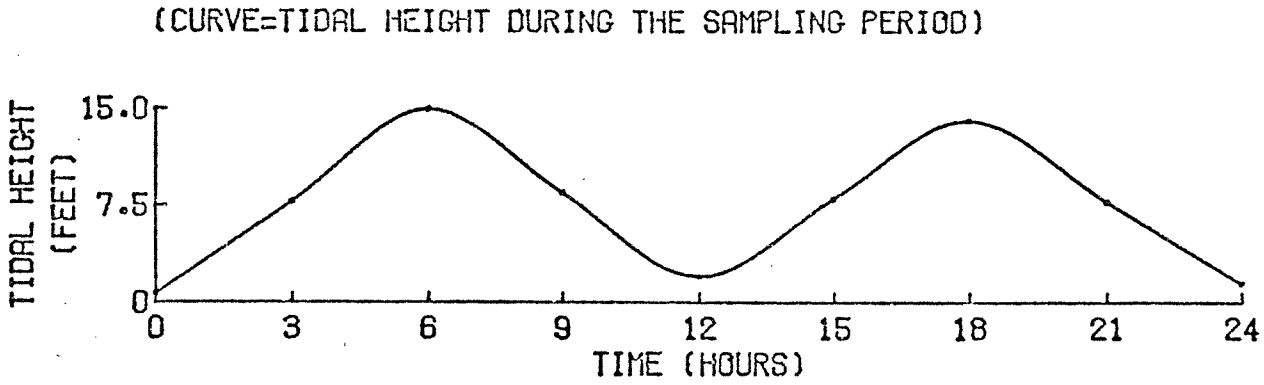


FIG. 13 . DISSOLVED OXYGEN CONCENTRATION AT STATIONS 1 THROUGH 7 IN 1974 MONTH OF GREATEST RANGE.



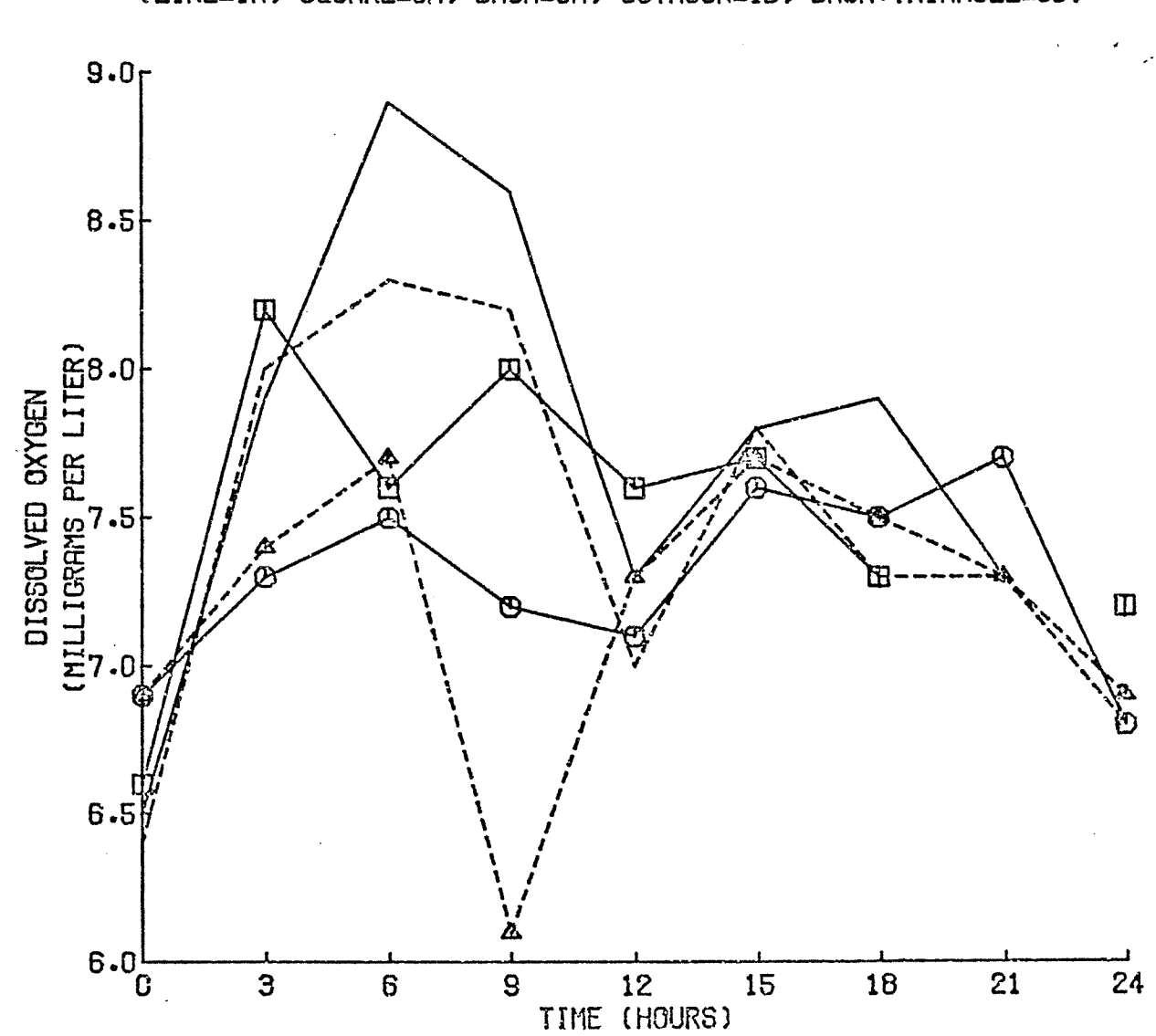


FIG. 14 . DISSOLVED OXYGEN CONC. AT STATIONS 1, 5, AND 6 IN HENDERSON INLET FROM NOON SEPT. 15 TO NOON SEPT. 16, 1974.

minimum occurring during the morning low tide (except at Station 6). The DO expressed as percent saturation followed the same pattern as the absolute DO (Fig. 15).

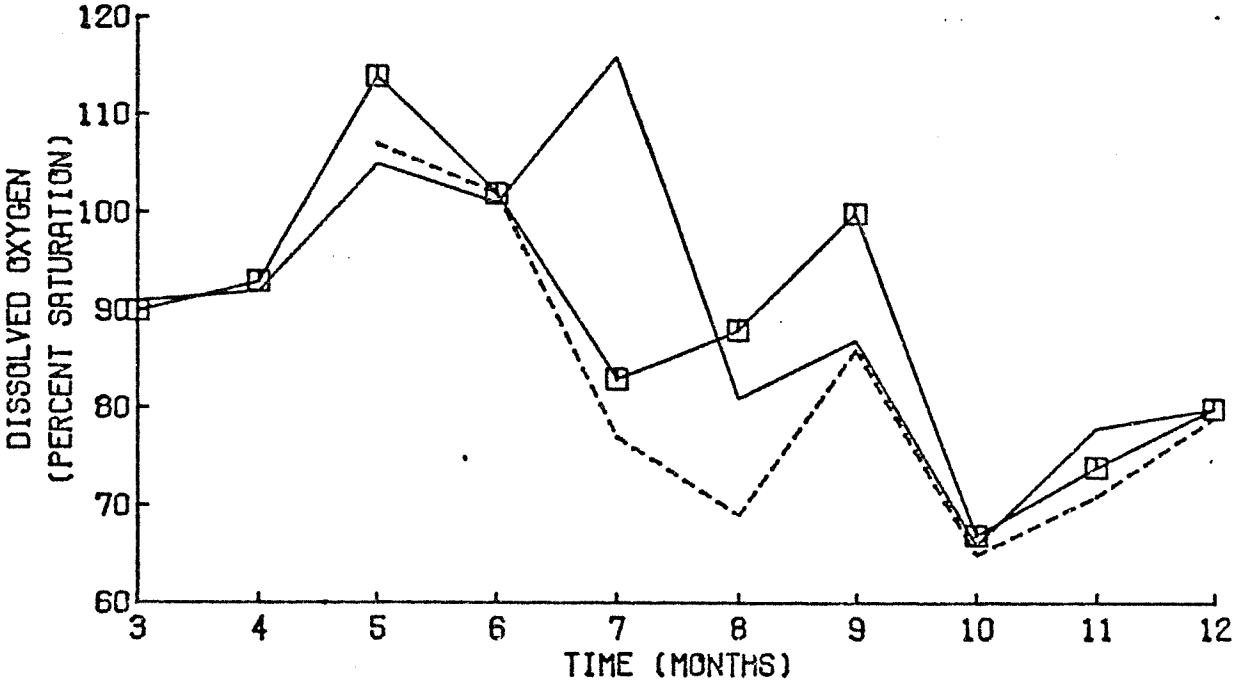
Ammonia

There were three peaks in the ammonia concentration at all stations at both the surface and bottom (Fig. 16). Each of the three ammonia peaks in the surface waters at the outer control occurred 1 month after each peak in chlorophyll a (Fig. 16A). The ammonia concentration in the surface waters inside the mariculture slip peaked 1 month after the April and September phytoplankton blooms, but also peaked during the July phytoplankton bloom. The highest ammonia concentration measured (212 µg/l as NH_3) was found near the surface at the high-density pens in July. The three ammonia peaks in the bottom waters occurred in June, August, and September at all stations (Fig. 16B). The highest ammonia concentrations were generally found within the mariculture slip (Fig. 17), except in August, which the highest concentration occurred near the bottom at the outer control. The summertime ammonia concentration was definitely affected by tidal factors and to a lesser degree by diurnal factors. The maximum ammonia concentrations occurred at low tide and the minima occurred near high tide (Fig. 18). Small peaks occurred during both day and nighttime high tides at Station 6A but only during the nighttime high tide at Station 1A.

Nutrients

The nitrate concentration steadily declined at all surface

A. (LINE=STATION 1A; DASH=STATION 5A; SQUARE=STATION 7A)



B. (LINE=STATION 1B; DASH=STATION 5B; SQUARE=STATION 7B)

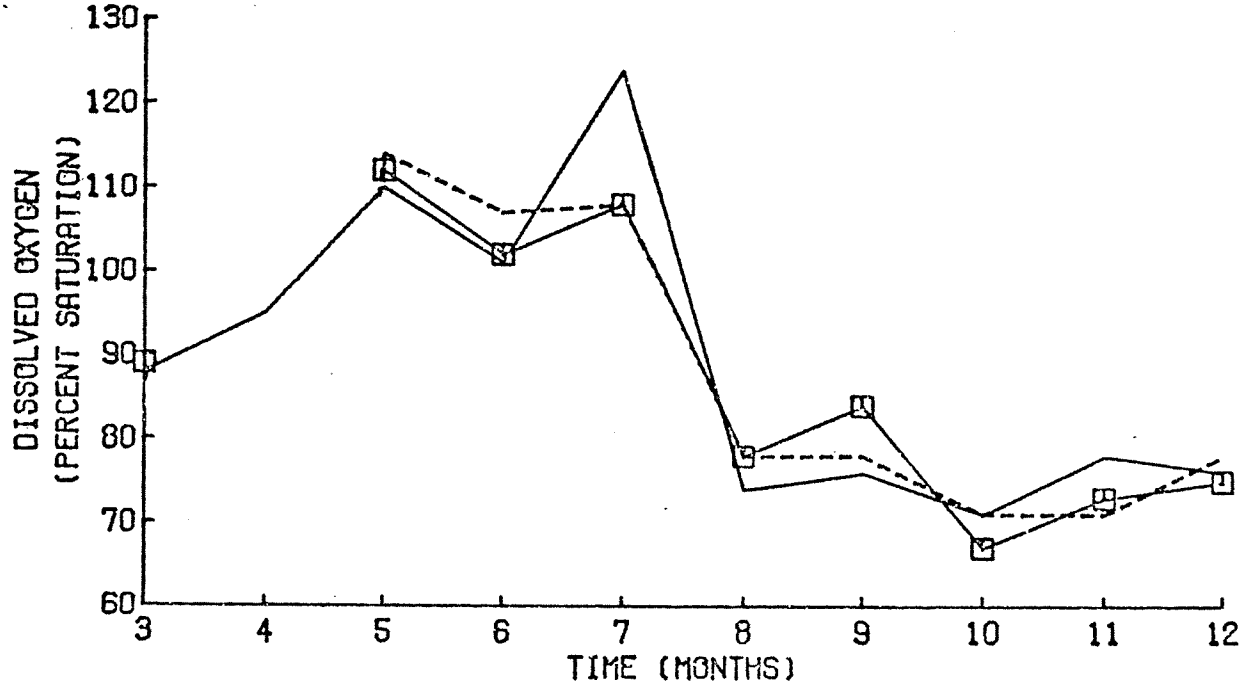
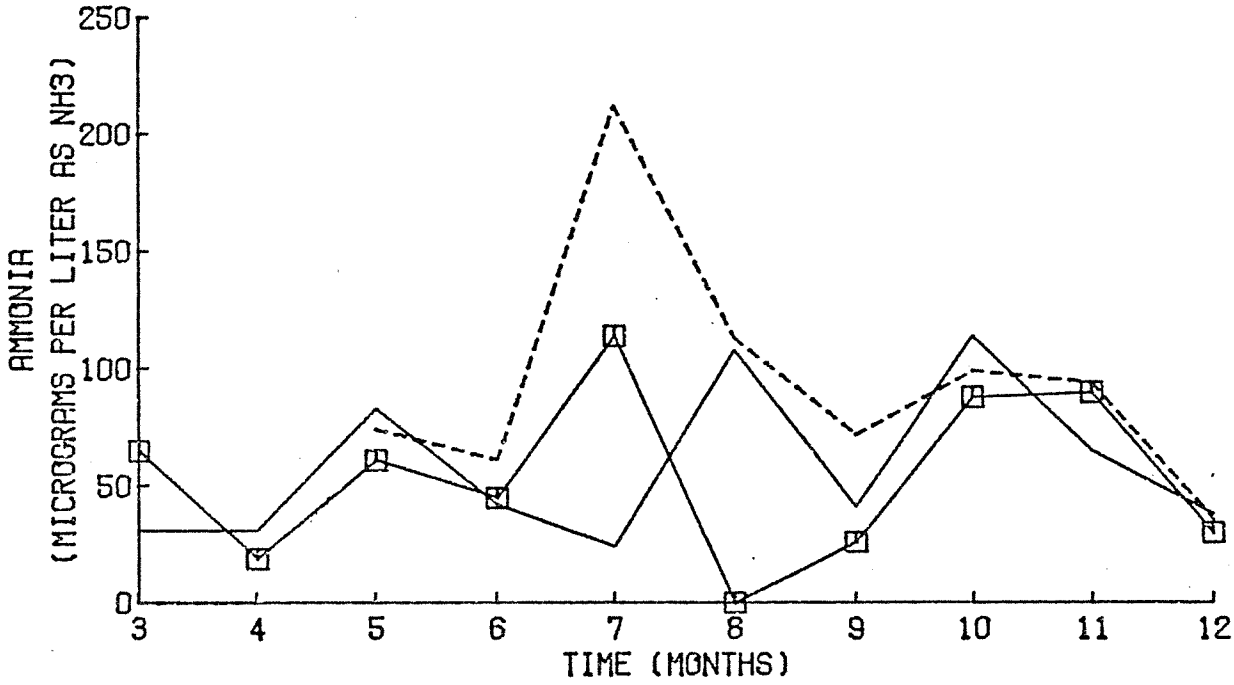


FIG. 15. DISSOLVED OXYGEN SATURATION AT STATIONS 1, 5, AND 7 AT HENDERSON INLET MARCH TO DECEMBER 1974.

A. (LINE=STATION 1A; DASH=STATION 5A; SQUARE=STATION 7A)



B. (LINE=STATION 1B; DASH=STATION 5B; SQUARE=STATION 7B)

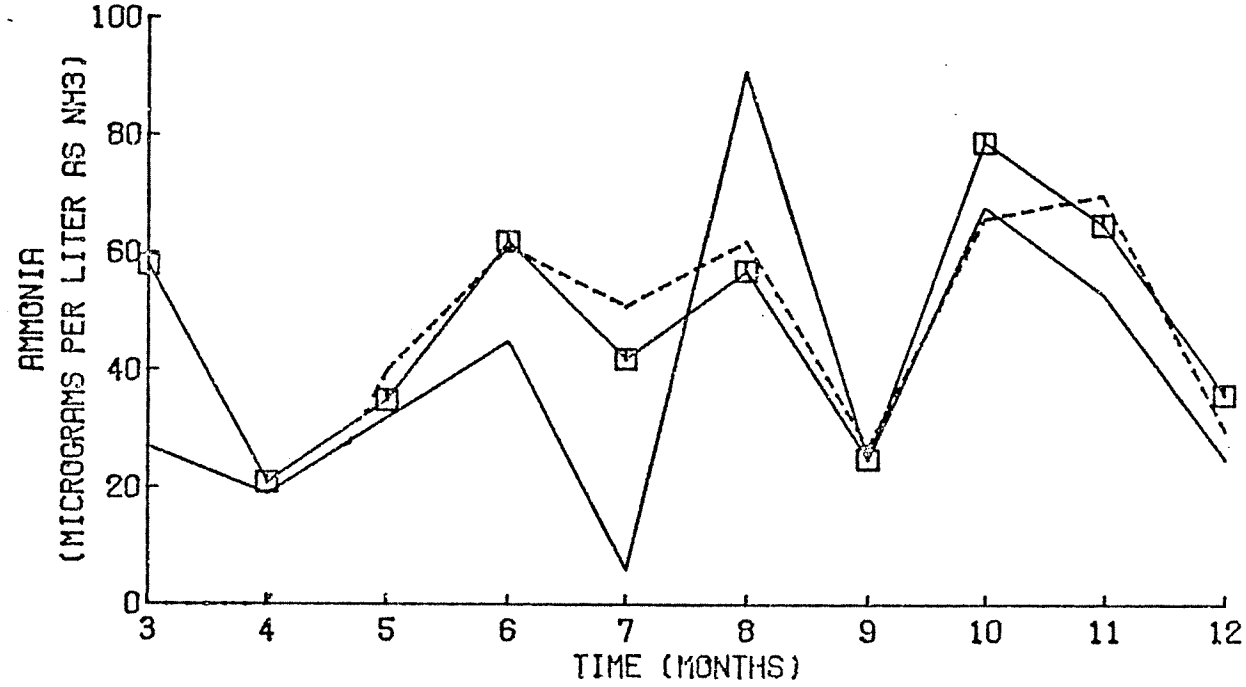
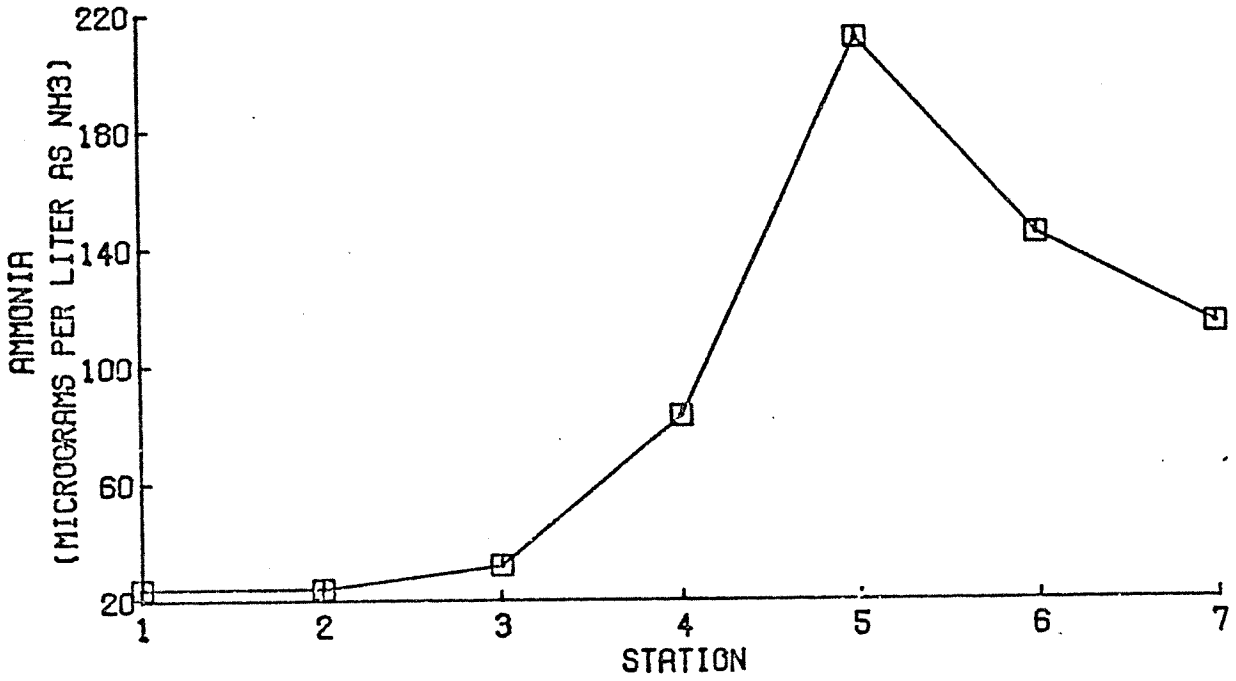


FIG. 16. AMMONIA CONCENTRATION AT STATIONS 1, 5, AND 7 AT HENDERSON INLET MARCH TO DECEMBER 1974.

A. 1M BELOW SURFACE IN JULY



B. 1M ABOVE BOTTOM IN JULY

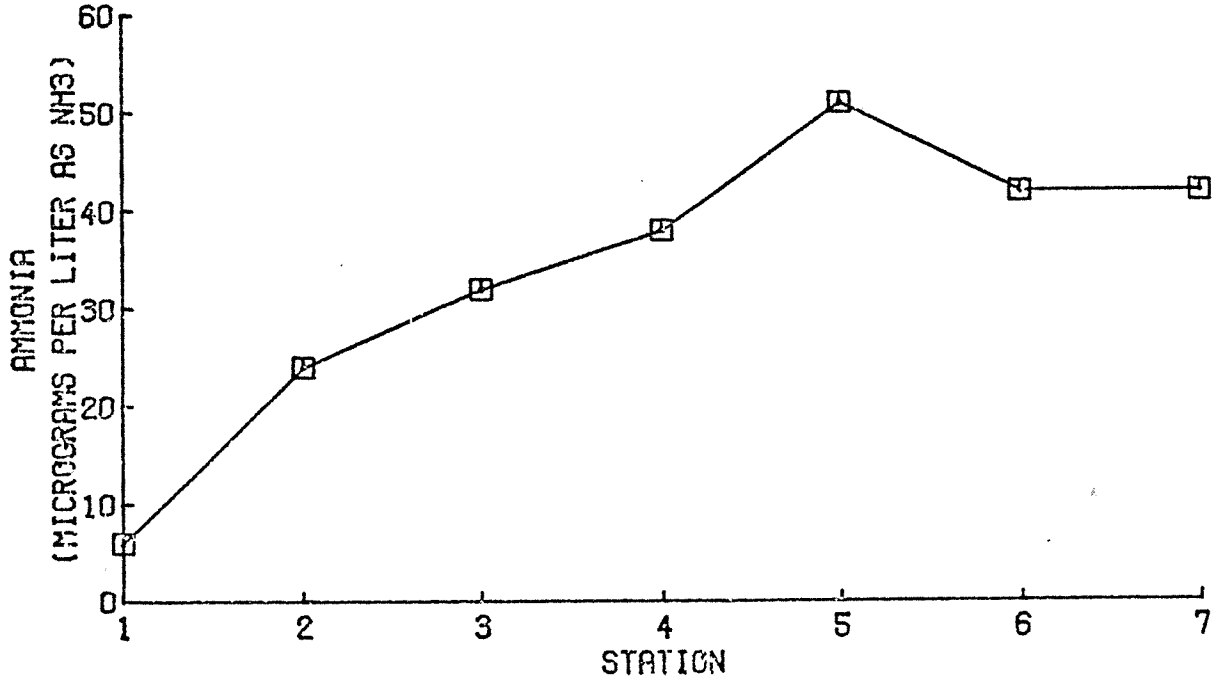
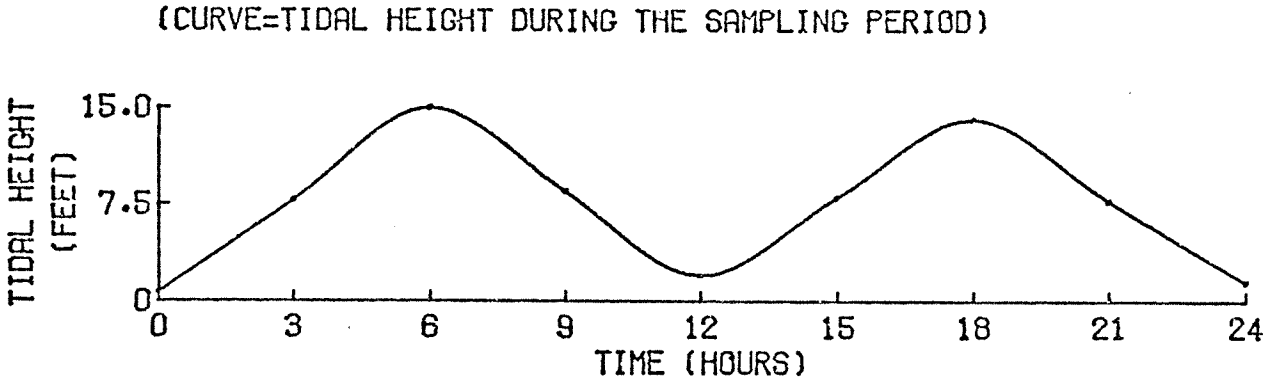


FIG. 17. AMMONIA CONCENTRATION AT STATIONS 1 THROUGH 7 IN 1974 MONTH OF GREATEST RANGE.



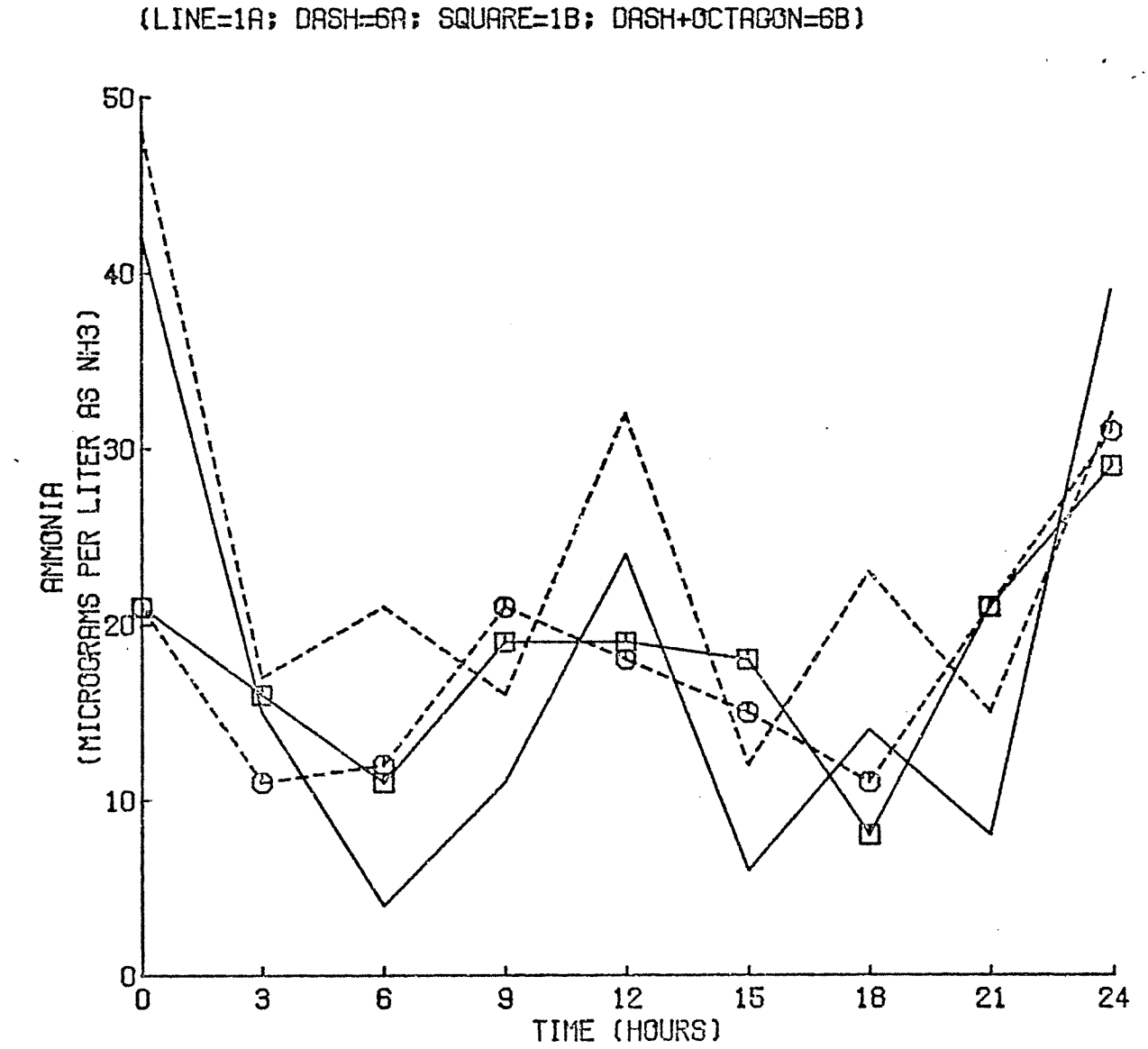


FIG. 18 . AMMONIA CONCENTRATION AT STATIONS 1 AND 6 IN HENDERSON INLET FROM NOON SEPT. 15 TO NOON SEPT. 16, 1974.

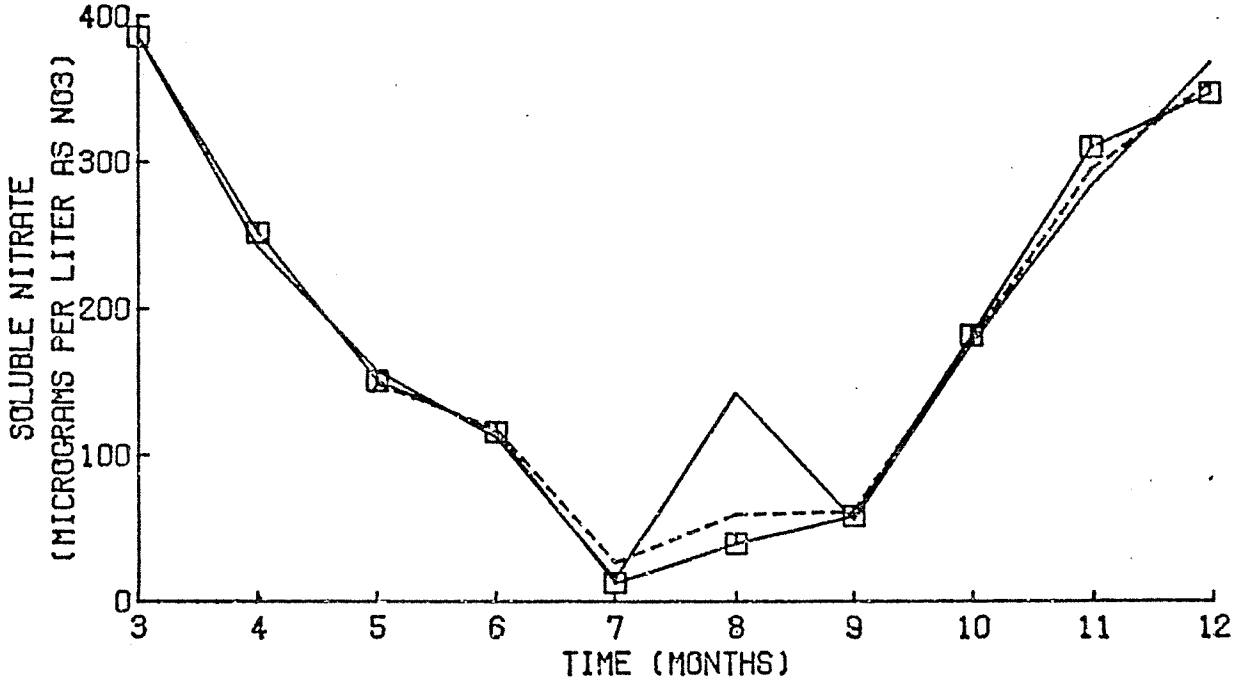
stations (Fig. 19A) from about 400 µg/l during the winter to near zero in April, then jumped slightly in August and declined in September, then steadily climbed back up to almost 400 µg/l again in December. In the bottom waters (Fig. 19B), the nitrate concentration followed the same general pattern except it didn't decline below 100 µg/l at the stations within the mariculture slip. Near the surface, the nitrate concentration was generally lower in the mariculture slip than at the outer control (Fig. 20A) except during the abrupt August peak. The concentration near the bottom was generally higher within the mariculture slip (Figs. 19B and 20 B).

The ortho-phosphate concentration generally followed the same pattern as the nitrate except that the ortho-phosphate reached a minimum in May instead of July and the concentrations in the surface waters of the mariculture slip were generally higher than outside the slip (Figs. 21A and 22A). The August ortho-phosphate sample from Station 5A was not included in Fig. 19A because it was apparently contaminated with phosphate. The total phosphorus concentration followed the same general trend as the ortho-phosphate (Fig. 23). The total phosphorus sample from Station 7A in September was apparently contaminated with phosphate also.

Total Organic Carbon

Four peaks in total organic carbon concentration were observed near the surface and bottom at all stations in April, July, September, and November (Fig. 24). As seen in Fig. 25, the highest concentration at the surface occurred near the low-density pen in August, and the

A. (LINE=STATION 1A; DASH=STATION 5A; SQUARE=STATION 7A)



B. (LINE=STATION 1B; DASH=STATION 5B; SQUARE=STATION 7B)

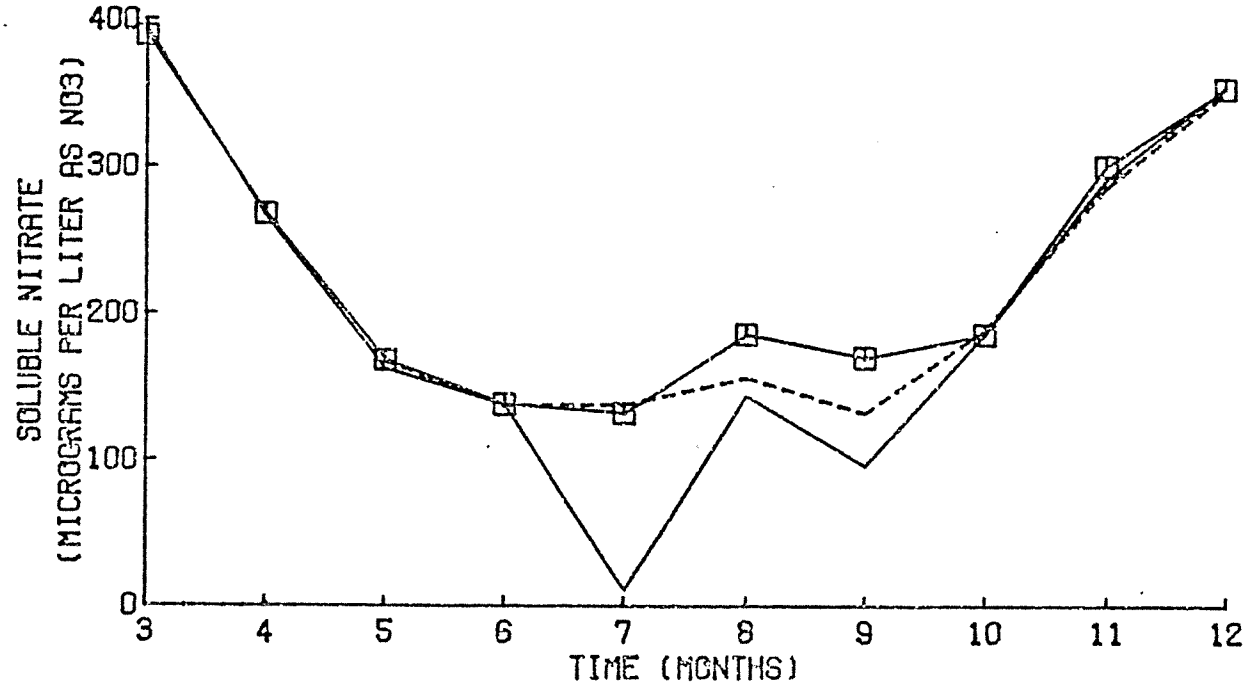
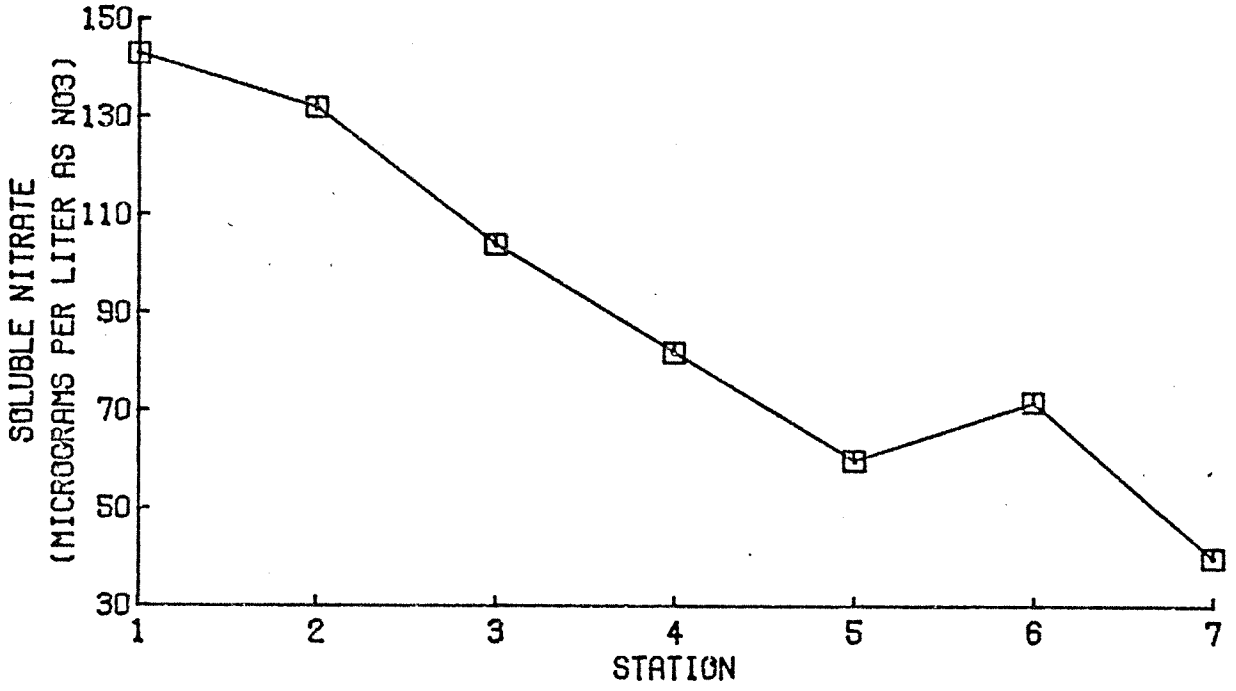


FIG. 19. SOLUBLE NITRATE CONCENTRATION AT STATIONS 1, 5, AND 7 AT HENDERSON INLET MARCH TO DECEMBER 1974.

A. 1M BELOW SURFACE IN AUGUST



B. 1M ABOVE BOTTOM IN JULY

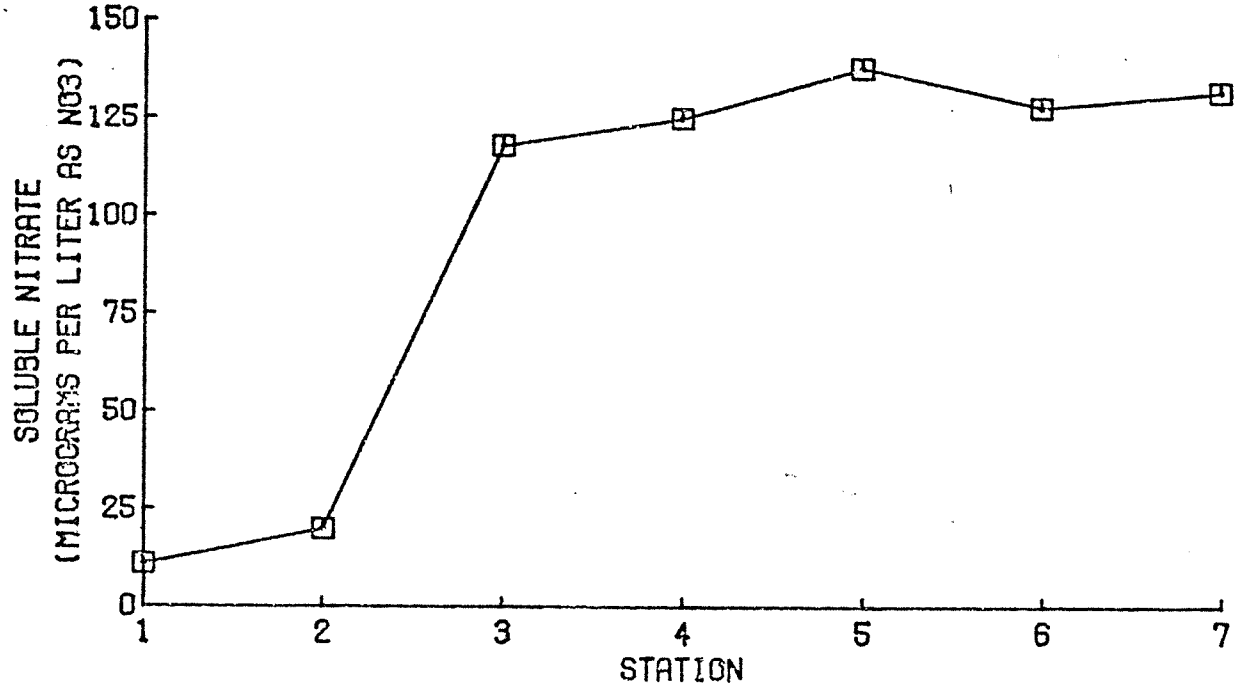
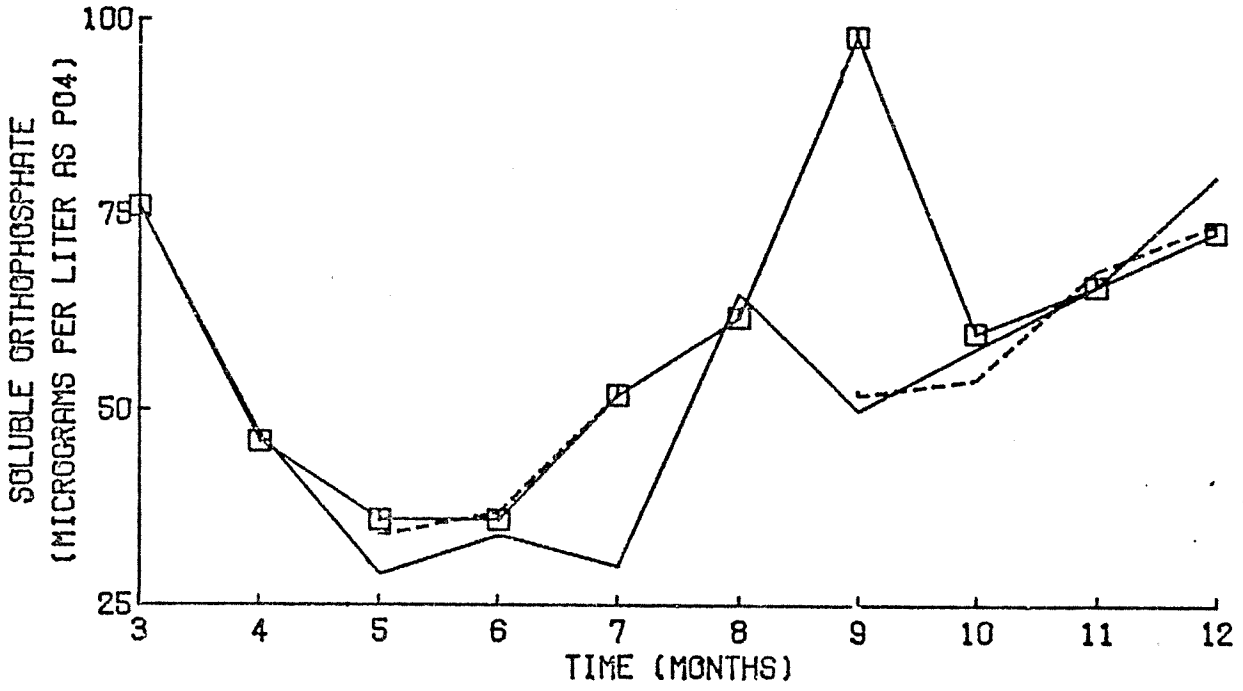


FIG. 20. SOLUBLE NITRATE CONCENTRATION AT STATIONS 1 THROUGH 7 IN 1974 MONTH OF GREATEST RANGE.

A. (LINE=STATION 1A; DASH=STATION 5A; SQUARE=STATION 7A)



B. (LINE=STATION 1B; DASH=STATION 5B; SQUARE=STATION 7B)

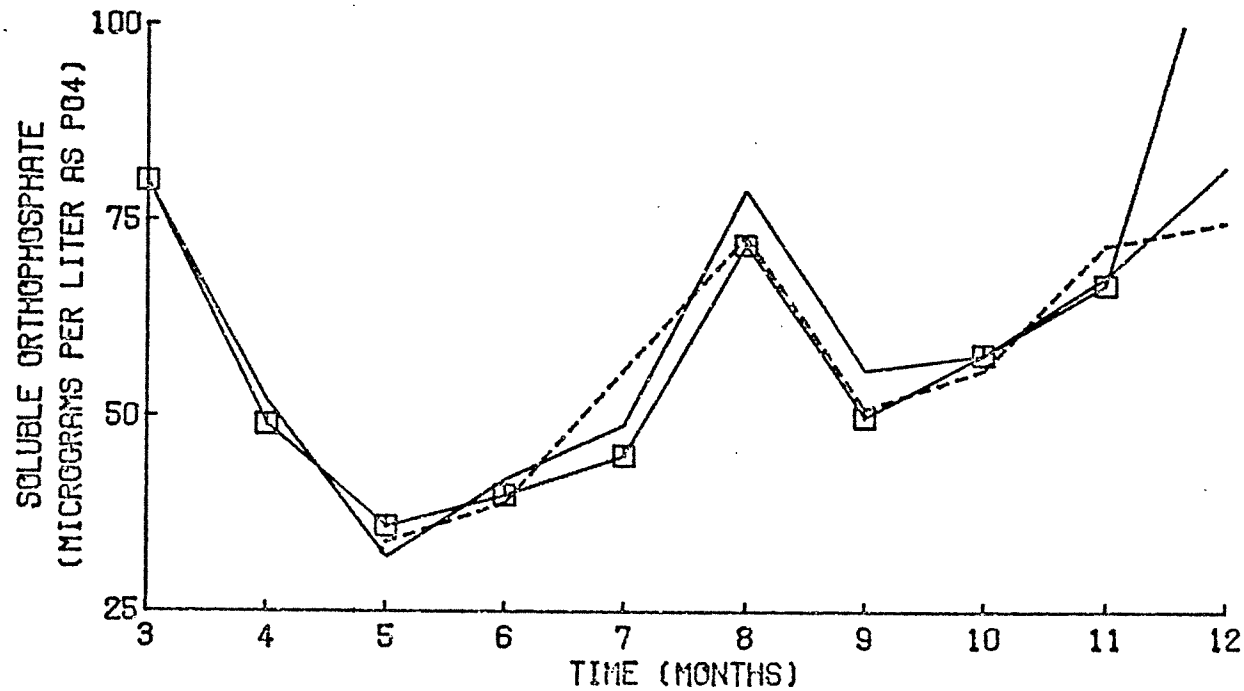
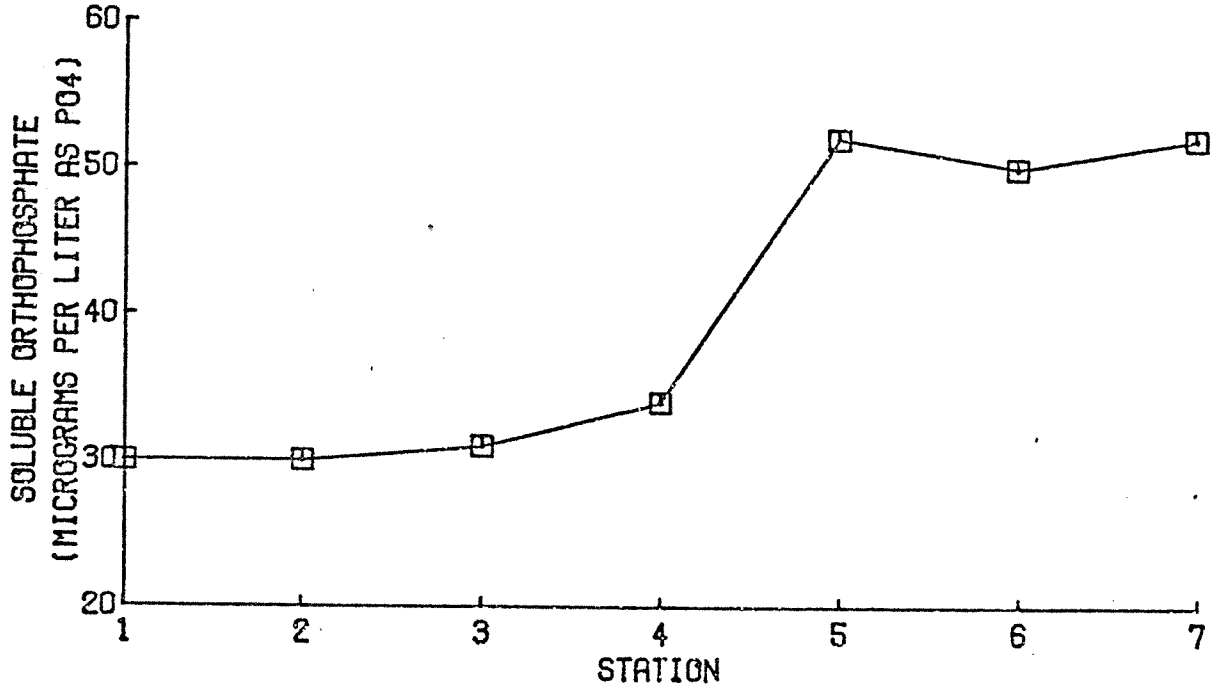


FIG. 21. SOLUBLE ORTHOPHOSPHATE CONCENTRATION AT STATIONS 1, 5, AND 7 AT HENDERSON INLET MARCH TO DECEMBER 1974.

A. 1M BELOW SURFACE IN JULY



B. 1M ABOVE BOTTOM IN DECEMBER

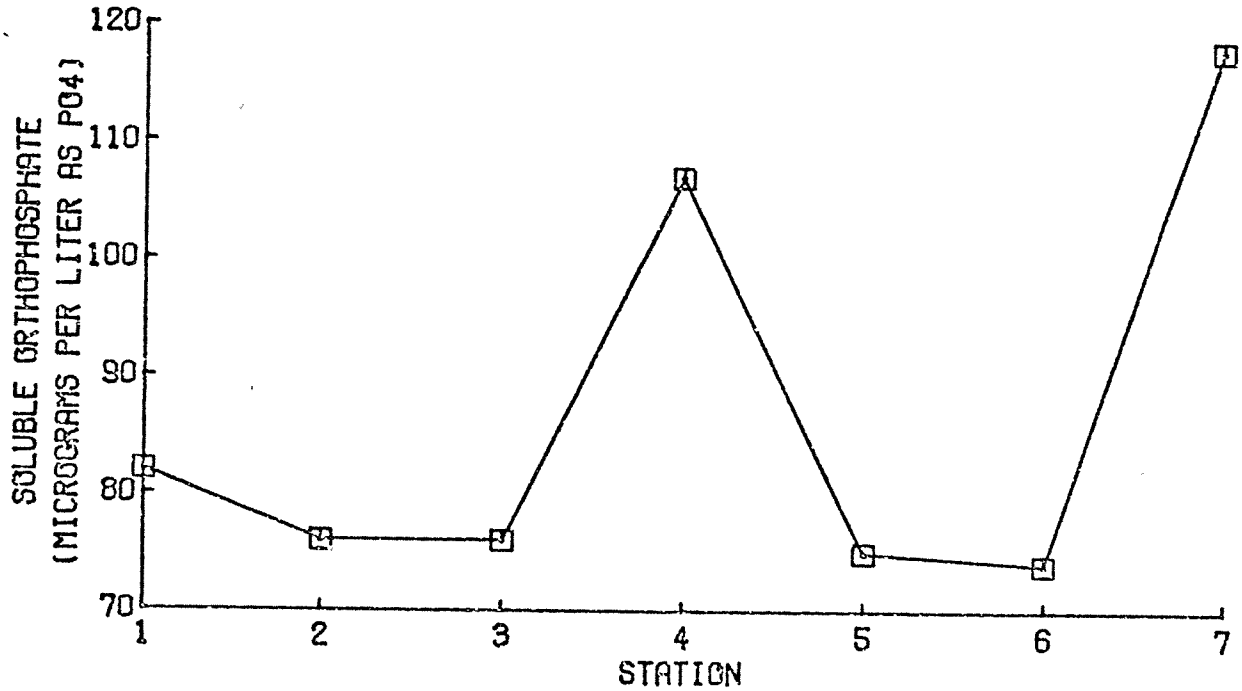
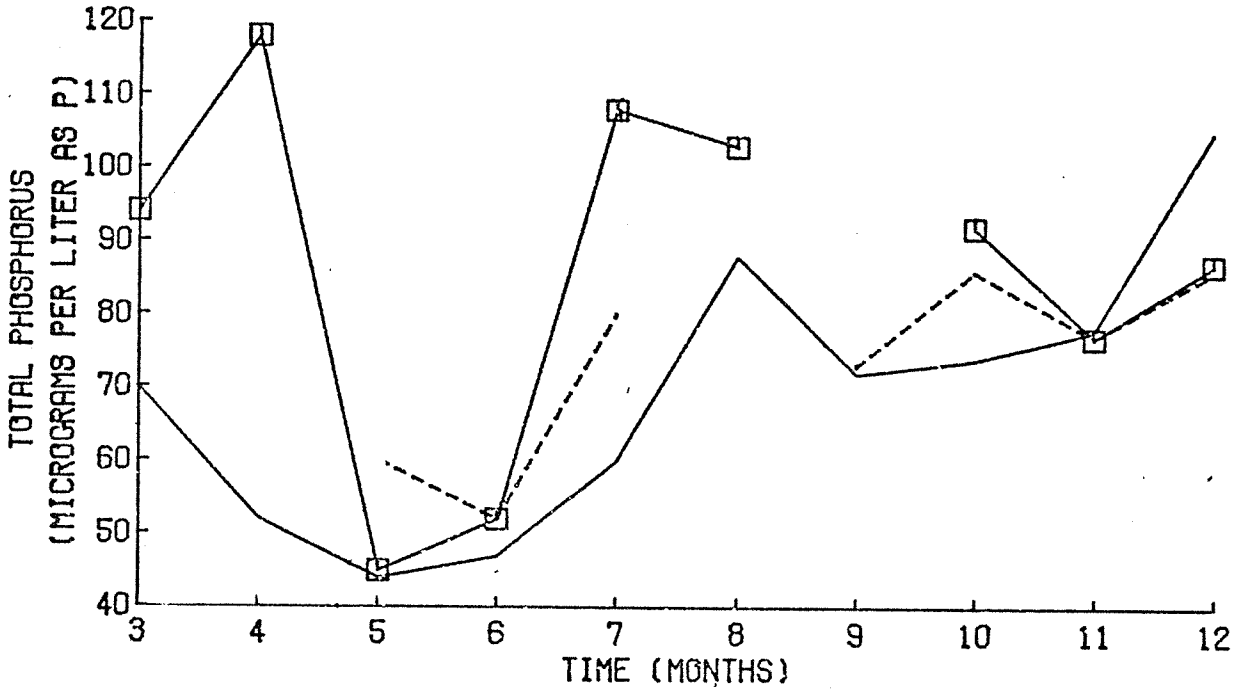


FIG. 22. SOLUBLE ORTHOPHOSPHATE CONCENTRATION AT STATIONS 1 THROUGH 7 IN 1974 MONTH OF GREATEST RANGE.

A. (LINE=STATION 1A; DASH=STATION 5A; SQUARE=STATION 7A)



B. (LINE=STATION 1B; DASH=STATION 5B; SQUARE=STATION 7B)

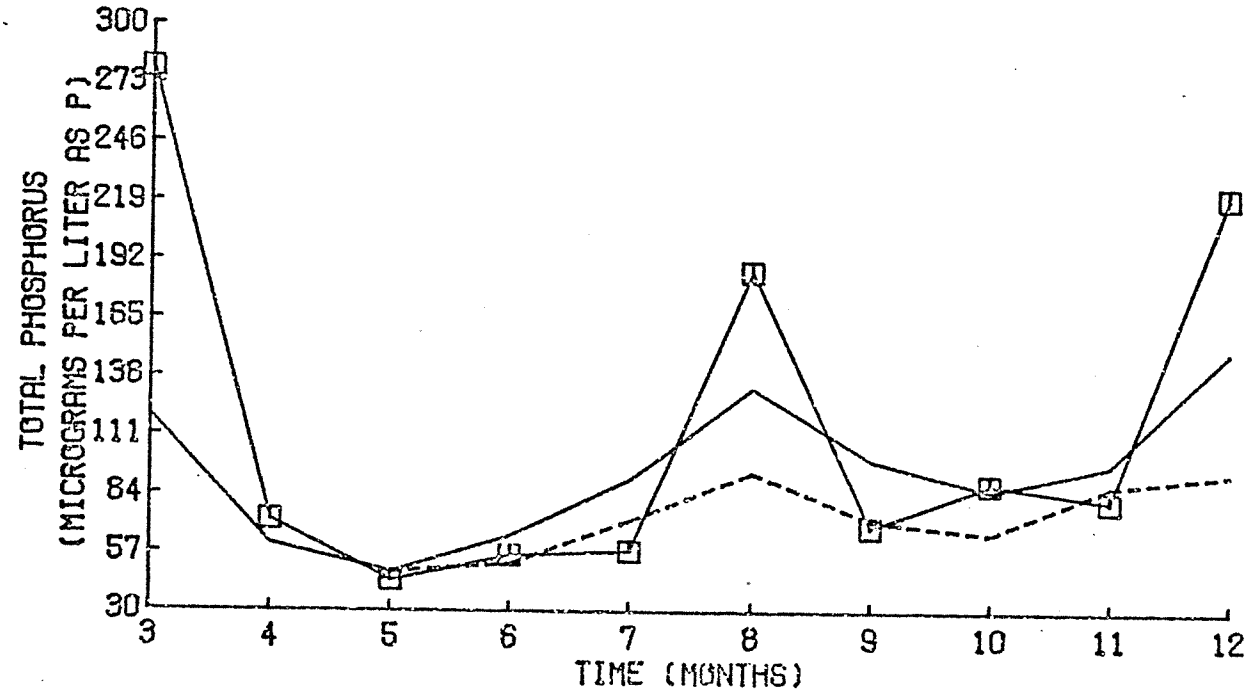
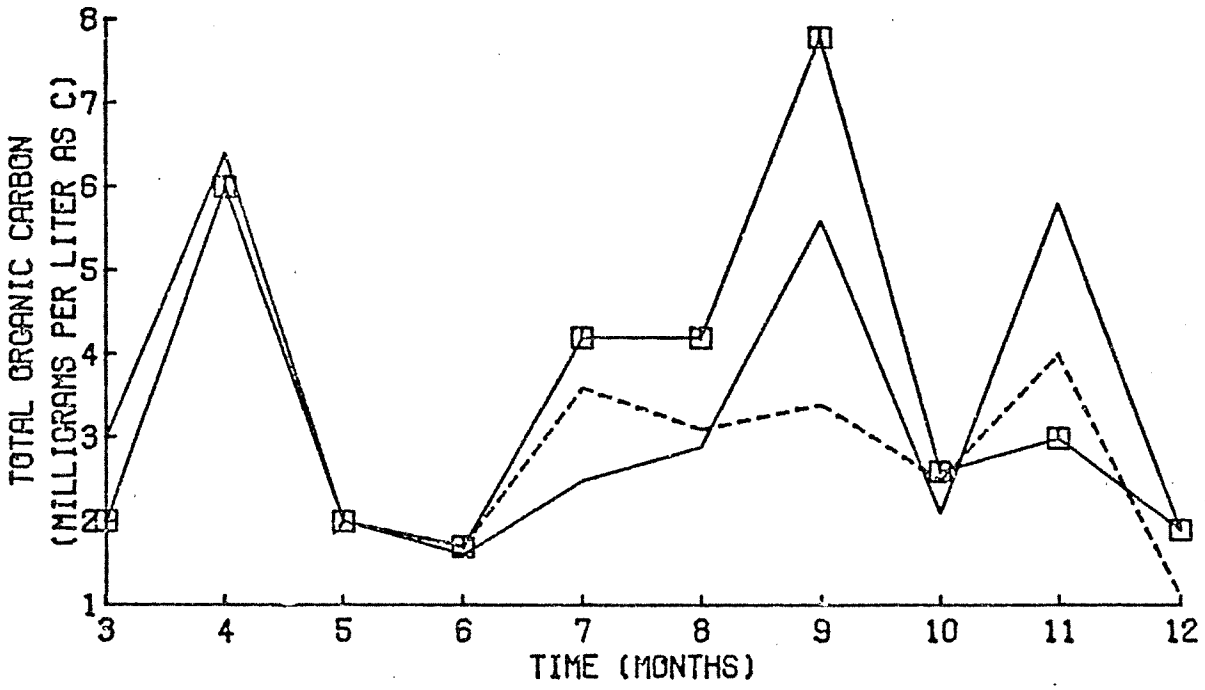


FIG. 23. TOTAL PHOSPHORUS CONCENTRATION AT STATIONS 1, 5, AND 7 AT HENDERSON INLET MARCH TO DECEMBER 1974.

A. (LINE=STATION 1A; DASH=STATION 5A; SQUARE=STATION 7A)



B. (LINE=STATION 1B; DASH=STATION 5B; SQUARE=STATION 7B)

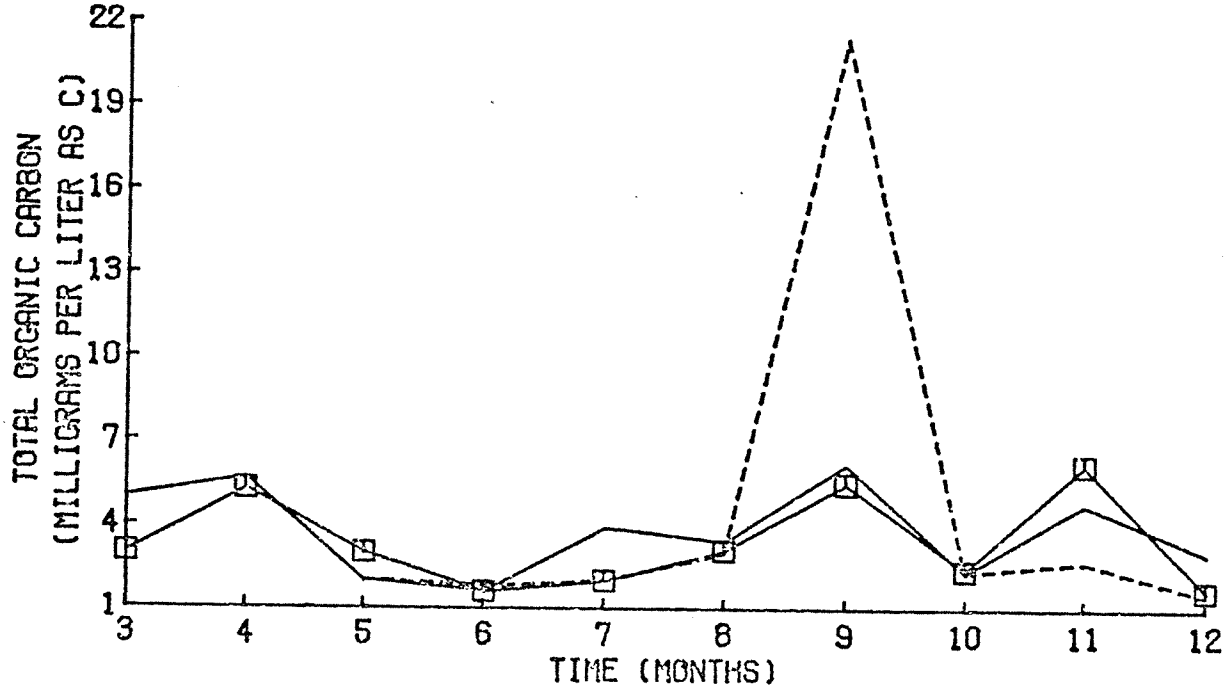
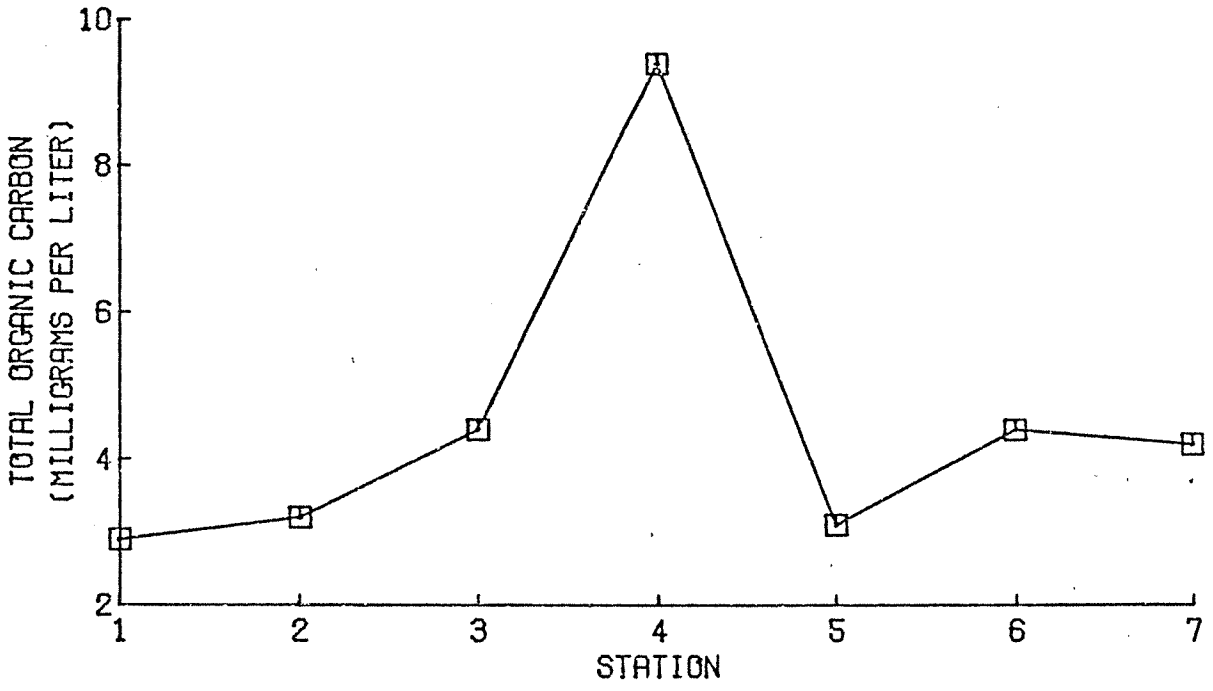


FIG. 24. TOTAL ORGANIC CARBON CONCENTRATION AT STATIONS 1, 5, AND 7 AT HENDERSON INLET MARCH TO DECEMBER 1974.

A. 1M BELOW SURFACE IN AUGUST



B. 1M ABOVE BOTTOM IN SEPTEMBER

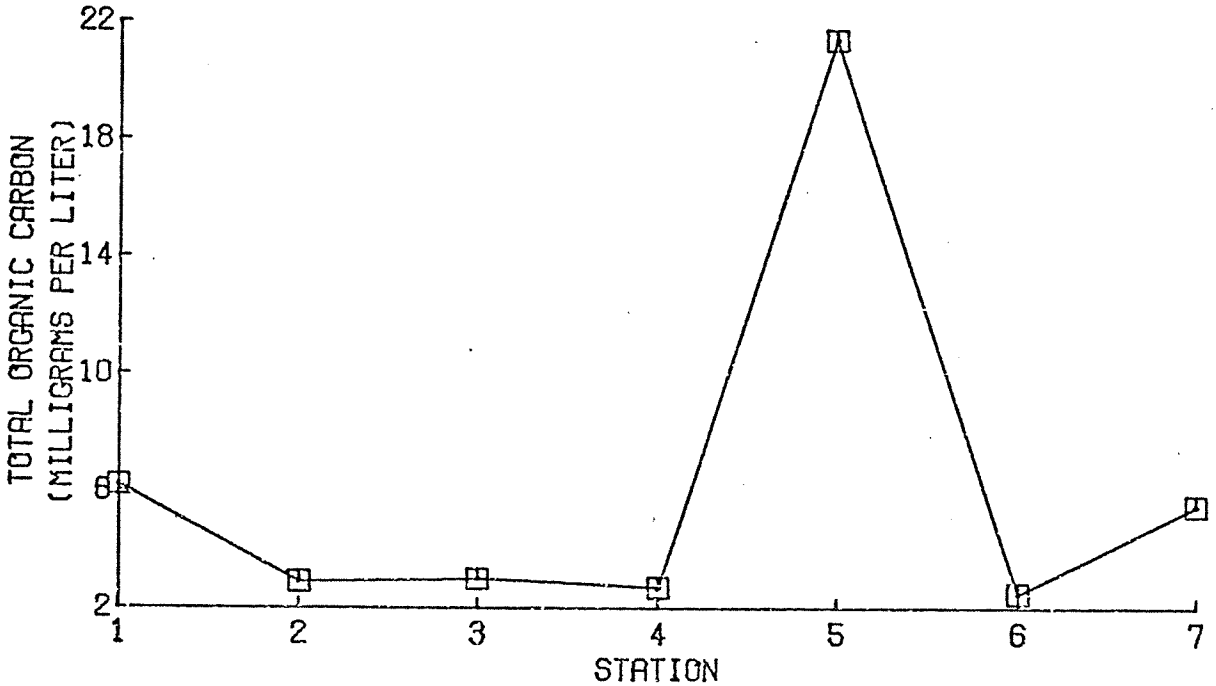


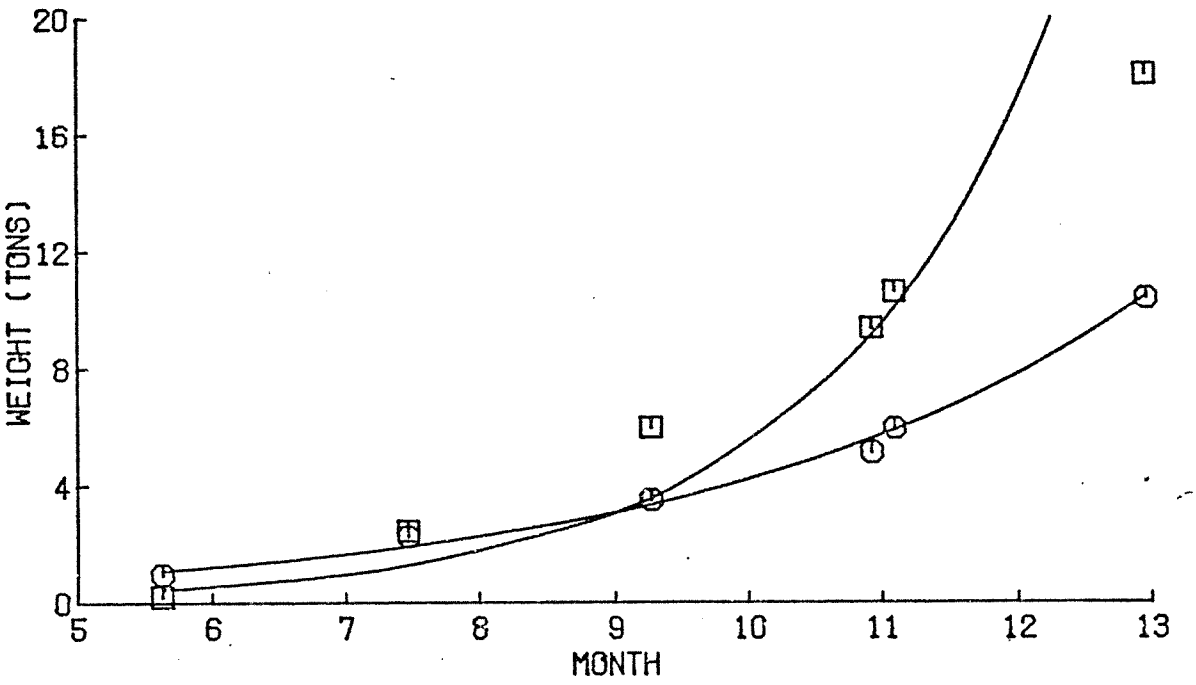
FIG. 25 . TOTAL ORGANIC CARBON CONCENTRATION AT STATIONS 1 THROUGH 7 IN 1974 MONTH OF GREATEST RANGE.

highest concentration at the bottom occurred near the high-density pens in September. The concentration near the surface was generally lower near the salmon pens than at either control station (Fig. 24A). The concentration was similar at all stations near the bottom, except when it increased near the high-density pens in August.

Fish Production

The amount of fish food introduced into the pens and the biomass of fish in the pens are important parameters to examine since they are directly related to the input of organic matter near the pens. A sample of OMP (fish food) was found to contain 7.7% nitrogen and 47.0% carbon. The cumulative weight of food and fish in the high- and low-density pens in 1974 is summarized in Fig. 26. Smooth curves were fitted by least squares, assuming a linear model after logarithmically transforming the weights. It appears that the fish biomass increased logarithmically with time, but the weight of fish food, which was regulated by the feeders, did not. There was a rapid increase in feeding rate between September and December in both pens. By December, over 18 tons of fish food had been introduced to the high-density pens (Float B) and 12 tons had been introduced to Float A. The mean overall conversion ratios $(\frac{\text{food wt}}{\text{fish wt}})$ for the fish in both the high- and low-density pens were close to two, indicating that much of the food did not get converted into fish biomass.

A. HIGH DENSITY PEN (SQUARE=FEED; OCTAGON=BIOMASS)



B. LOW DENSITY PEN (SQUARE=FEED; OCTAGON=BIOMASS)

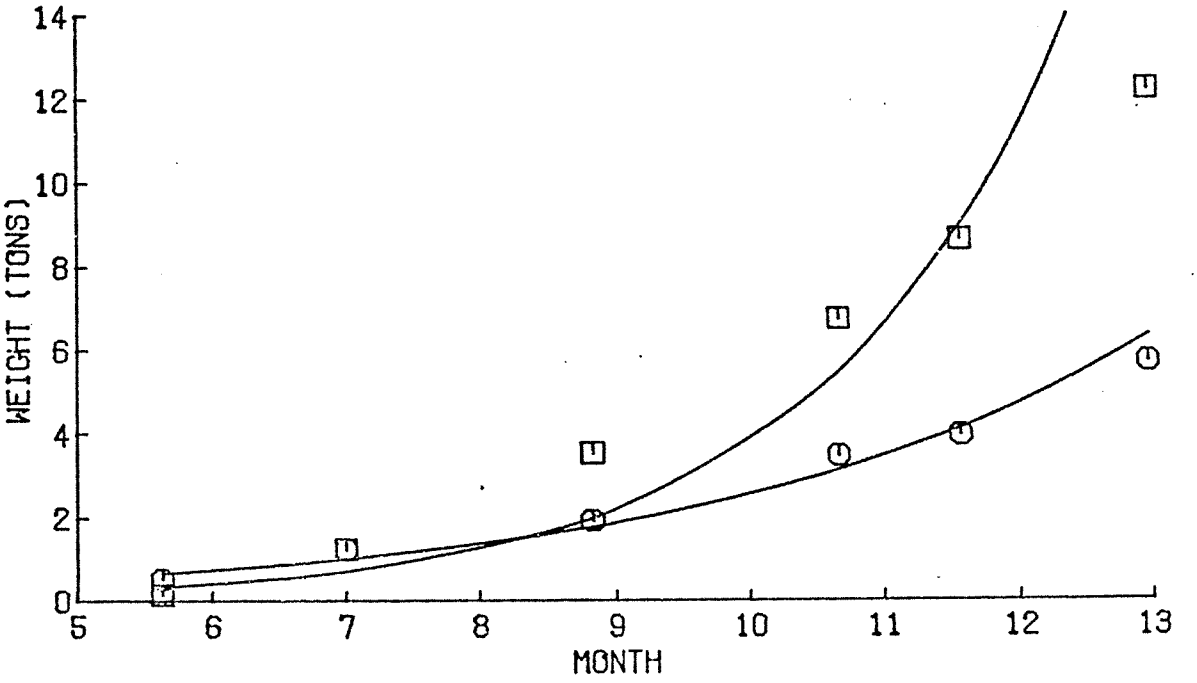


FIG. 26. CUMULATIVE WEIGHT OF FEED AND FISH IN THE HIGH AND LOW DENSITY PENS MAY TO DECEMBER 1974.

Sediments

The carbon concentration, expressed as percent by weight, in the core samples taken from each transect in 1974 is summarized in Table 1. The mean carbon concentrations and standard deviations at Transects 1, 3, and 5 are plotted in Fig. 27. Analysis of variance with the Newman-Keuls multiple range test ($\alpha = 0.05$) was used to determine which of these means were significantly different with respect to time and location. The mean carbon concentration at Transect 5 was significantly lower than the mean at Transects 1 and 3 April through December. The mean concentration at Transect 3 was not significantly different from the mean at Transect 1 in April and June, but was significantly higher than the mean at both control transects in August and December.

No significant change in mean carbon concentration was observed at Transect 5 from April to December. The carbon concentration at Transect 1 increased significantly from April to June but not from June to December. At Transect 3, the carbon concentration did not change significantly from April to June but increased significantly from April to August and from August to December.

Linear regression models were also calculated for the carbon concentrations at Transects 1, 3, and 5. The best fitting regression lines (based on correlation coefficient and F value) are plotted in Fig. 28. A logarithmic transformation of carbon concentration ($\ln y = 1.23 + 0.10 x$) is the best fitting model for the carbon concentration as a function of time at Transect 3. Simple linear

Table 1. Mean carbon concentration (percent by weight) ± standard deviation in 1974 sediment cores

Month	Transect 1	2	3	4	5
April	5.0 ± 0.6	6.6 ± 1.4	5.7 ± 1.2	6.1 ± 0.5	3.8 ± 0.3
June	6.1 ± 0.5	5.6 ± 0.4	6.4 ± 0.5	6.1 ± 0.6	4.1 ± 0.4
August	5.7 ± 1.3	7.8 ± 1.3	8.1 ± 2.0	7.4 ± 1.4	3.8 ± 0.1
December	5.6 ± 0.2		11.3 ± 0.8		3.9 ± 0.3

(SQUARE=TRANSECT 1; OCTAGON=TRANSECT 3; TRIANGLE=TRANSECT 5)
(LINES=ST. DEV. OF TRANSECT 1; DASH=ST. DEV. OF TRANS. 3 AND 5)

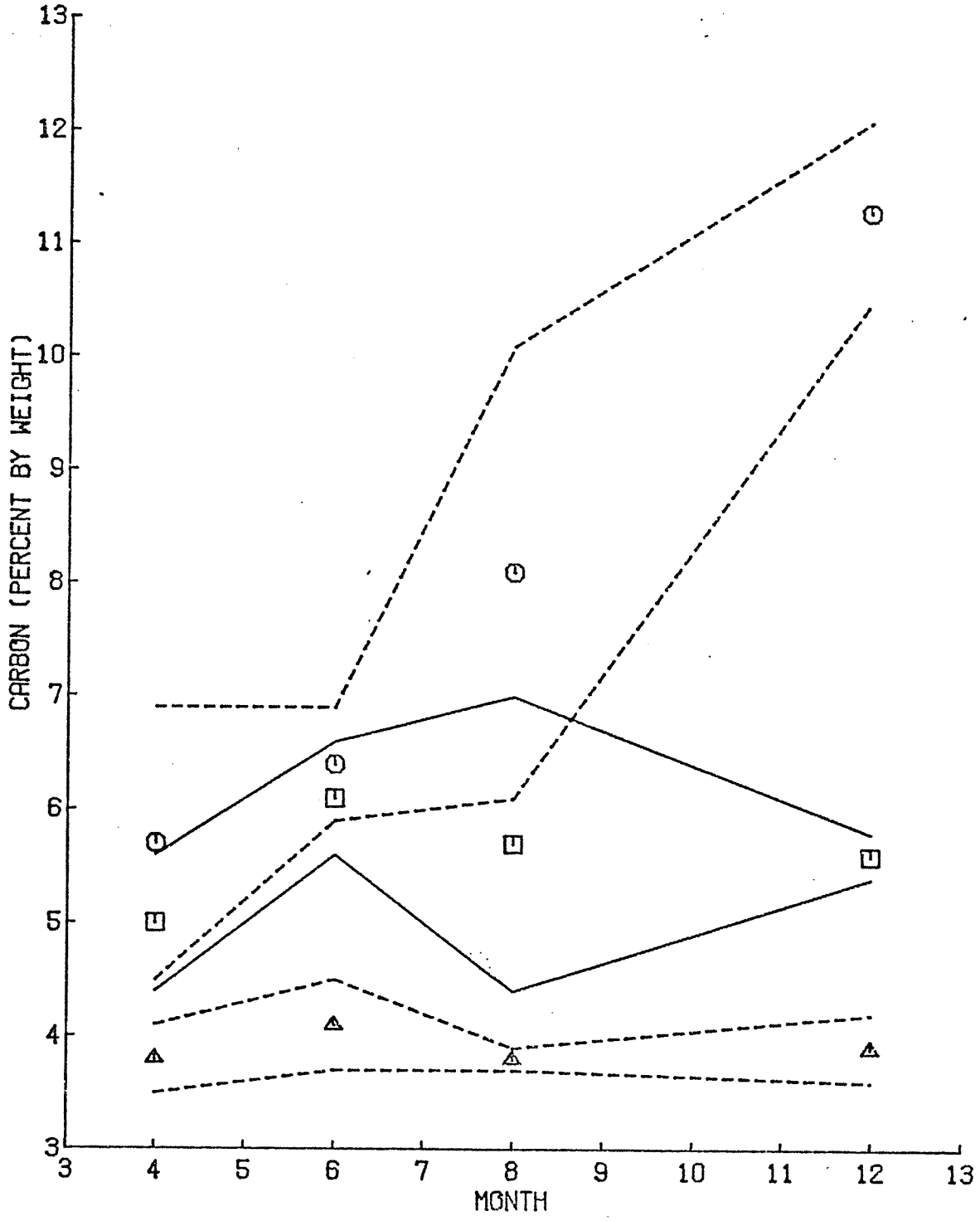


FIG. 27. MEAN CARBON CONCENTRATION AND STANDARD DEVIATIONS IN CORES FROM TRANSECTS 1, 3, AND 5 APRIL TO DECEMBER 1974.

(SQUARE=TRANSECT 1; OCTAGON=TRANSECT 3; TRIANGLE=TRANSECT 5)
(POINT=TRANSECT 4; LINES=REGRESSION LINES FOR TRANSECTS 1,3,5)

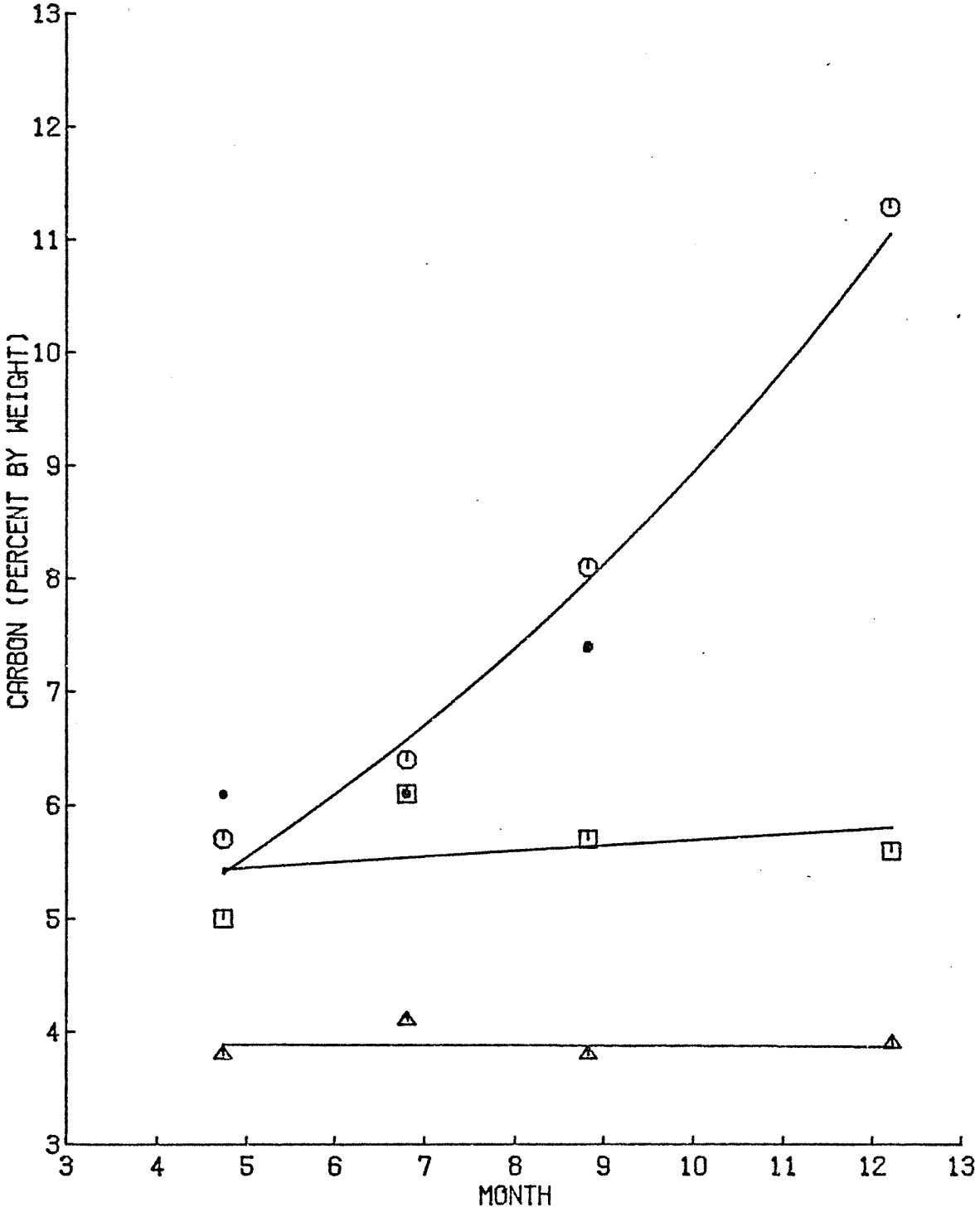


FIG. 28. MEAN CARBON CONC. AND REGRESSION LINES FROM TRANSECTS 1, 3, AND 5 WITH MEAN CARBON CONC. AT TRANSECT 4 IN 1974.

models with slopes statistically equal to zero (using the student t test at = 0.05; Zar, 1974) provided the best fit for carbon concentration at Transects 1 and 5. The mean carbon concentration at Transect 4 in Fig. 28 follows the same trend as the concentration at Transect 3.

The nitrogen concentration, expressed as percent by weight, in the core samples taken from each transect is summarized in Table 2. The mean nitrogen concentration and standard deviations at Transects 1, 3, and 5 are plotted in Fig. 29. Differences between mean nitrogen values were analyzed with the same statistical techniques described above for carbon.

The mean nitrogen concentration at Transect 5 was significantly lower than the mean at Transects 1 and 3 in all months sampled except August, when it was equal to the mean at Transect 1. The mean nitrogen concentration at Transect 3 was not significantly different from the mean at Transect 1 in April but was significantly higher than the mean at both control transects June through December. There was no significant change in mean nitrogen concentration from April to August at either control transect, but a significant increase was observed at both transects in December. At Transect 3, the mean nitrogen concentration increased significantly from April to June; then no significant change occurred from June to December.

Linear regression models were also calculated for the nitrogen concentrations at Transects 1, 3, and 5. The best fitting regression lines are plotted in Fig. 30. The best fitting model for the nitrogen concentration at Transect 3 described a logarithmic approach to an asymptote ($\ln (0.68-y) = 0.26-0.36x$). Simple linear models provided

Table 2. Mean nitrogen concentration (percent by weight) ± standard deviation in 1974 sediment cores.

Month	Transect				
	1	2	3	4	5
April	0.36 ± 0.03	0.39 ± 0.00	0.36 ± 0.02	0.34 ± 0.03	0.27 ± 0.00
June	0.40 ± 0.03	0.45 ± 0.05	0.59 ± 0.03	0.43 ± 0.06	0.25 ± 0.02
August	0.35 ± 0.15	0.57 ± 0.15	0.64 ± 0.15	0.46 ± 0.15	0.27 ± 0.02
December	0.45 ± 0.05		0.66 ± 0.13		0.33 ± 0.03

(SQUARE=TRANSECT 1; OCTAGON=TRANSECT 3; TRIANGLE=TRANSECT 5)
(LINES=ST. DEV. OF TRANSECT 1; DASH=ST. DEV. OF TRANS. 3 AND 5)

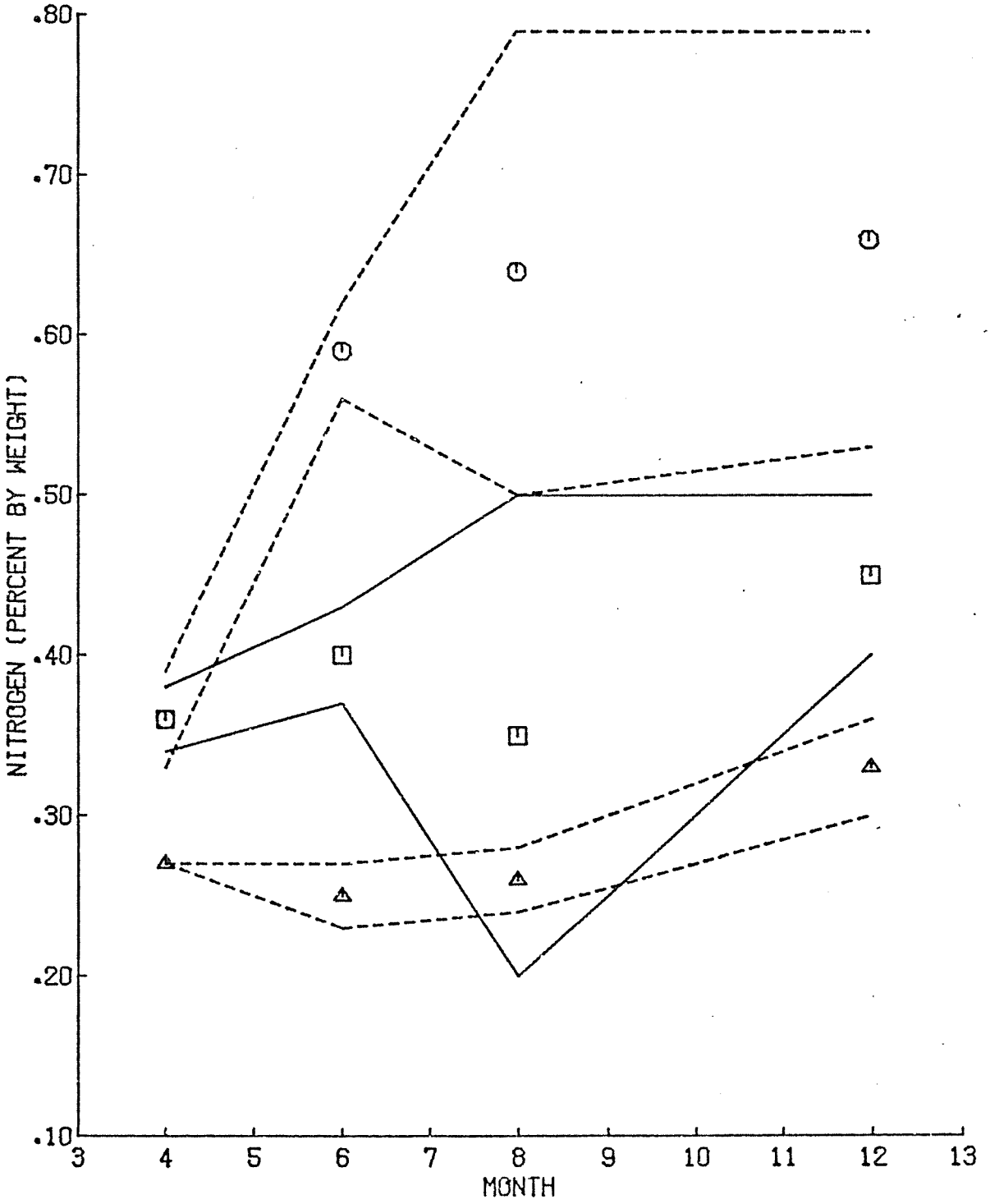


FIG. 29. MEAN NITROGEN CONCENTRATION AND STANDARD DEVIATIONS IN CORES FROM TRANSECTS 1, 3, AND 5 APRIL TO DECEMBER 1974.

(SQUARE=TRANSECT 1; OCTAGON=TRANSECT 3; TRIANGLE=TRANSECT 5)
(POINT=TRANSECT 4; LINES=REGRESSION LINES FOR TRANSECTS 1,3,5)

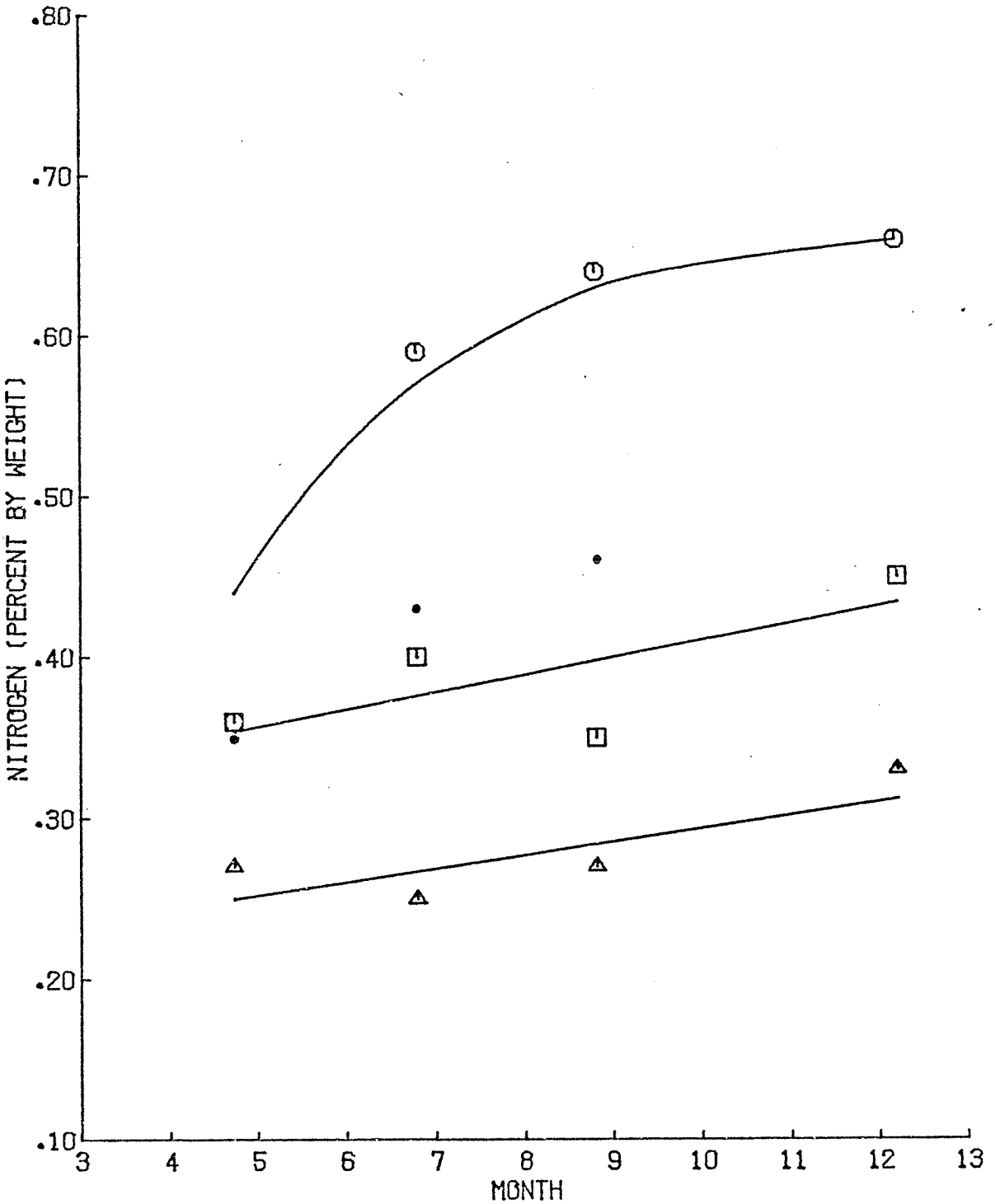


FIG. 30. MEAN NITROGEN CONC. AND REGRESSION LINES FROM TRANSECTS 1, 3, AND 5 WITH MEAN NITROGEN CONC. AT TRANSECT 4 IN 1974.

the best fit for nitrogen concentration at Transects 1 and 5. The slopes of the regression lines for these transects were significantly greater than zero but not significantly different from each other (using the student t test at $\alpha = 0.05$). The mean nitrogen concentrations at Transect 4 in Fig. 30 follow the same trend as the concentrations at Transect 3.

The carbon/nitrogen ratios at Transects 1, 3, and 5 are plotted in Fig. 31. Generally, there was an increase in the ratio at the control transects in June and August, with a concurrent decrease at Transect 3.

Benthic Communities

Epifauna

The benthic epifauna observed along the transects in 1974 are summarized in Table 3. The limitations of the technique should be considered before analyzing the data. It should be obvious that the technique is subjective and lacks precision. Individuals of sedentary species such as Metridium and Stylatula were recounted each month. Sampling error, recruitment, death, and a certain unknown amount of motility account for the monthly variation in the numbers of these sedentary species. Statistical techniques are not readily applicable to the relatively small numbers, but general trends are readily observable.

Evasterias and Cancer generally dominated the epifauna community within the mariculture slip, while Stylatula dominated at the outer control. The abundance of Evasterias was fairly constant at Transect 1 and 2 from May through December, but declined noticeably

(SQUARE=TRANSECT 1; OCTAGON=TRANSECT 3; TRIANGLE=TRANSECT 5)
(LINES=ST. DEV. OF TRANSECT 1; DASH=ST. DEV. OF TRANS. 3 AND 5)

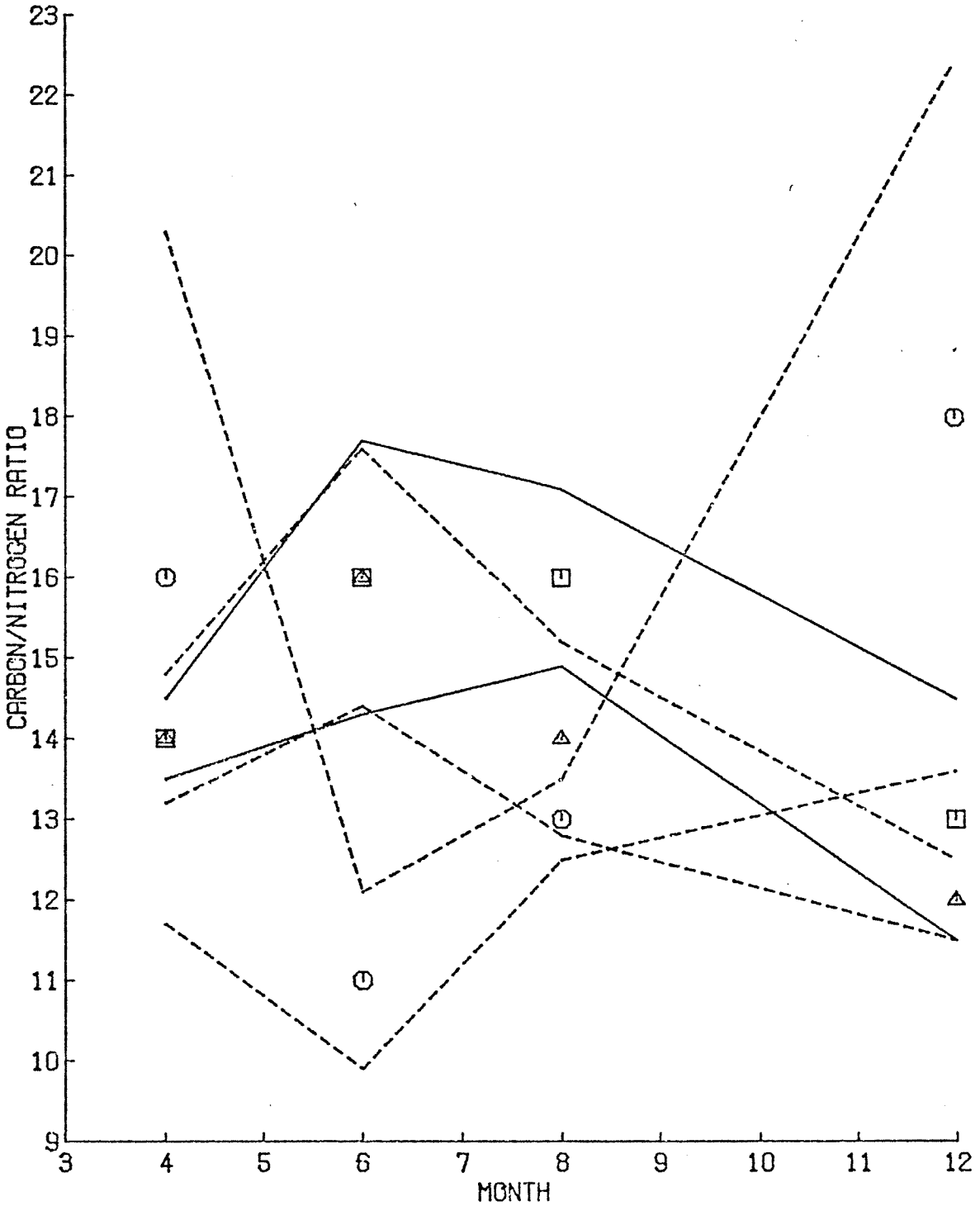


FIG. 31 . MEAN CARBON/NITROGEN RATIO AND STANDARD DEVIATIONS IN CORES FROM TRANSECTS 1, 3, AND 5 FROM APRIL TO DECEMBER 1974.

Table 3A. Epifauna observed along Transect 1, May to December 1974 (see Table 3F for further identification of organisms).

Organism	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
Evasterias	5	2	4	4	1	3	1	2	22
Cancer			4	2	1		3	1	11
Metridium		1	1	2	1	1		1	7
Eupentacta					2	2	2		6
Myoxocephalus			2						2
Lepidopsetta				1				1	2
Platichthys			1						1
Parastichopus					1				1
Pagurus				1					1
Pugettia						1			1
Total Specimens	5	3	12	10	6	7	6	5	48
Total Species	1	2	5	5	5	4	3	4	10

Table 3B. Epifauna observed along Transect 2, May to December 1974 (see Table 3F).

Organism	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
Evasterias	13	9	5	3	3	3	7	4	47
Cancer	1		1	9	7	1	5		24
Lepidopsetta			1	4	2				7
Eupentacta		2	2		1				5
Parastichopus					1	1		1	3
Oregonia	2								2
Pugettia				2					2
Hermissenda			1		1				2
Metridium	1								1
Pagurus		1							1
Myoxocephalus		1							1
Total Specimens	17	12	10	18	15	5	12	5	95
Total Species	4	4	5	4	6	3	2	2	11

Table 3C. Epifauna observed along Transect 3, May to December 1974 (see Table 3F).

Organism	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
Cancer			5	22	15	8	4	4	58
Evasteria	32	8	3	4	1	1	3	1	53
Metridium	1	2	1	1	1	2	1	1	10
Myoxocephalus	1					2	1	4	8
Parastichopus	2		2	3					7
Eupentacta	3	1	1	1	1				7
Leptocottus			1			1	2		4
Lepidopsetta			1			1	1		3
Oregonia				1					1
Pugettia			1						1
Total Specimens	39	11	15	32	18	15	12	10	152
Total Species	5	3	8	6	4	6	6	4	10

Table 3D. Epifauna observed along Transect 4, May to December 1974 (see Table 3F).

Organism	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
Cancer	1	9	9	18	12	2			51
Evasterias	2	7	10	4	6	2		1	32
Metridium	1		1	1	1	1		1	6
Lepidopsetta		1	1					2	4
Myoxocephalus	1						1	1	3
Platichthys							1	2	3
Eupentacta					1				1
Total Specimens	5	17	21	23	20	5	2	7	100
Total Species	4	3	4	3	4	3	2	5	7

Table 3E. Epifauna observed along Transect 5, May to December 1974 (see Table 3F).

Organism	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
Stylatula	21	29	41	29	30	42	25	26	243
Cancer			1	1	1	1			4
Pagurus				2				1	3
Evasterias				1				1	2
Leptocottus			1						1
Crago			1						1
Total Specimens	21	29	44	33	31	43	25	28	254
Total Species	1	1	2	4	2	2	1	3	6

Table 3F. Identification of epifauna observed along transects.

Scientific name	Common name
Cancer gracilis	Small cancer crab
Crago nigricauda	Sand shrimp
Eupentacta sp.	White sea cucumber
Evasterias troschellii	Common 5-rayed starfish
Hermissenda crassicornis	Irridescent nudibranch
Lepidopsetta bilineata	Rock sole
Leptocottus armatus	Staghorn sculpin
Metridium senile	Sea anemone
Myoxocephalus polyacanthocephalus	Great sculpin
Oregonia sp.	Decorator crab
Pagurus sp.	Hermit crab
Parastichopus californicus	Spiny sea cucumber
Platichthys stellatus	Starry flounder
Pugettia sp.	Kelp crab
Stylatula elongata	Sea pen

during this period at Transects 3 and 4. Cancer gracilis started appearing at all transects in July, became increasingly abundant until August and September, then progressively less abundant through December. C. gracilis was noticeably more abundant at the transects under the salmon pens than at any of the control transects. The fish (Myoxocephalus, Lepidopsetta, Platichthys, and Leptocottus) as a group, were also more abundant at the transects under the salmon pens than at any of the control transects. The maximum number of individuals and species at all transects was generally found during July, August, and September (the months of maximum temperature and salinity).

While sampling the epifauna in July, thin patches of white material were observed along the transects under the salmon pens but not at the control transects. The white material was examined microscopically and found to be a filamentous, white, sulfide-oxidizing bacteria (Beggiatoa sp.; Breed, et al, 1948). Further sampling showed that Beggiatoa was also present at the control transects but in much lower numbers. Patches of Beggiatoa were observed under the salmon pens from July through December. Visible patches of Beggiatoa were never observed at the control transects.

Infauna

The benthic infauna species sampled at all transects in 1974 are summarized in Table 4. A total of 16,195 specimens belonging to 91 species was sampled. The 41 species forming 98% of the total abundance were identified to genus and species. The other 50 species were not identified but are distinct species (except the Platyheminthes and

Table 4. Benthic infauna sampled at Transects 1 through 5 in 1974.

Organism	4/22					6/24					8/25					12/7			Total
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	3	5	
Phylum Annelida																			
Class Polychaeta																			
Nephtys cornuata franciscana	222	120	121	137	259	139	111	77	102	182	238	237	40	140	180	109		52	2442
Capitella capitata			2	1		2		328	25			1	366	162			866		1753
Pholoe minuta	1	1	5	44	68	5	6	7	26	99	9	6	10	9	170	8		114	588
Prionospio pinnata	8	18	7	6	3	18	16	11	8	1	13	12	1	3	3	27	3	15	173
Gyptis brevipalpa			2	8	5	3	6	8	5	2	7	5	20	19	2	12	47	3	154
Spiophanes berkeleyorum			2	4	25	7	7	9	8	15	11	5		3	15	7		9	127
Prionospio cirrifera			1	11	26	9		7	3	12	3	3	7	2	12	5	5	17	123
Cossura longocirrata	5		11	30	4	17	4	3	17	2	4	2			7				107
Armandia brevis	3	5	5	1	1	6	4	14	6			1		5		6	4	6	103
Glycinde picta	5	7	3	3		10	3	6	6	2	6	8	6	8	5	4		2	84
Dorvillea rudolphi	5	1	2			1		2	3		5		1	10			35		65
Thoryx parvus			2	1	3	15	12	2		1	2		2		2			1	43
Terebellides stroemi			5	4	3	4	5	1	3		3		1		2				31
Nereis sp.				1		4	1	2	1				3	4	1	5	3	1	26
Otopsis longipes	3		1	3	1		1	1	2	3		1		1	4	1	2	1	25
Eteone longa	2		1		4	1					5	3	1	1	1	3		2	24
Lumbrineris luti			2	1	2		2		4		1	1	1		1			4	19
Polychaete species 1					3					3					5			8	19
" " 2	1				3										8			4	16
" " 3	1		3	3		1	1	1	4		1								15
" " 4			1			3	3		1	2	1				1				12
" " 5			2	1	2	3	2	1								1			12
" " 6		2		2			3		2		2			1					12
" " 7							1			2	1				5			2	11
" " 8								1			1					1		6	9
" " 9			2				1				2	1		1		1			8
" " 10							1					1			3	1			6
" " 11			1	1				1		1	1	1							6
" " 12											1				1	2			4

Table 4, cont'd

Organism	4/22					6/24					8/25					12/7			
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	3	5	Total
Polychaete species 13						1	1			2									4
" " 14								2										1	3
" " 15					2					1									3
" " 16							1				1				1				3
" " 17											2	1							3
" " 18	2				1														3
" " 19			1						1		1								3
" " 20				1		1													2
" " 21	1																		1
" " 22												1							1
" " 23													1						1
" " 24			1																1
" " 25				1															1
" " 26						1													1
" " 27								1											1
" " 28											1								1
" " 29							1												1
Phylum Arthropoda																			
Class Crustacea																			
Eudorella pacifica	420	197	180	122	2117	565	726	166	240	1100	248	243		18	1115	93		757	8307
Pinnixa schmitti		1		17	39	6	3	5	7	27	4	4	10	17	72	1		47	260
Protomedeia articulata	3	4	1	5	1	36	19	2	1	8	6	1	3	2	18	3	2	42	157
Heterophoxus oculatus			2	11	10		1	7	16	15	3	1	3	1	2	1		8	81
Amphipod species 1			3		6				2	7					5			4	27
Amphipod species 2					2		1		1	5					8			8	25
Crago nigricauda						1	3		4	2	7	1			5	1			24
Amphipod species 3					17														17
Isopod species 1								4			1		5						10
Nebalia sp.													4	1			1		6
Amphipod species 4					2	1				1								1	5
Isopod species 2							1	1											2
Amphipod species 5			1							1									2
Amphipod species 6						1	1												2

Table 4, cont'd

Organism	4/22					6/24					8/25					12/7			
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	3	5	Total
Cancer gracilis														1					1
Pagurus sp.				1															1
Lophopanopaeus sp.						1													1
Cumacean species 1											1								1
Phylum Mollusca																			
Class Gastropoda																			
Nassarius mendicus	59	44	30	47	10	44	46	61	74	8	53	44	49	91	12	54	4	11	741
Odostomia tacomaensis	2	43	27	15	4	18	13	15	17	4	16	14		8	4	17		3	221
Mitrella gouldii	3		1	1	1	2	3	9	1		1	2		7	4				35
Alvania compacta						1			2		1	9	1	9		9			32
Sulcoretusa sp.	1		1	1		2	1									1		1	8
Acteocina sp.										2									2
Nudibranch species 1																3			3
Class Pelecypoda																			
Mysella tumida		3	1	2	2	4	8	16	2	9	17	13	26	16	14	12	8	7	165
Immature *Macomas*	10	15	6	10	6	9	5	1			9	54	2	3	15	16	2	2	165
Macoma nasuta	4	4	6	3	4	8	10	8	4	9	8	9	3	8	14	3	5	5	115
Psephidia ovalis		1	1	1	18			1	2	31				1	23			7	86
Axinopsida serricatus			4		1	4	1		1		4			2	1				18
Clinocardium nuttalli		1		1	1	1			3					1	2	1		1	12
Macoma incongrua								1	1		4		2	1			1		10
Parvilucina tenuisculpta	1			2	2	1					1		1						8
Macoma inquinata								1						1		2	1		5
Protothaca staminea			1				1	1	1			1							5
Macoma inconspicua									1				1	2					4
Macoma yoldiformis		1		1		1													3
Thyasira sp.						1				1						1			3
Macoma alaskana					1						1								2
Macoma brota	1																		1
Yoldia sp.																			1

Table 4, cont'd

Organism	4/22					6/24					8/25					12/7			Total
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	3	5	
Phylum Echinodermata																			
Amphiodia urtica			3	12	27	3	1	2	11	29	2	3	3	3	34			31	161
Eupentacta sp.																1			1
Phylum Coelenterata																			
Metridium senile							1				1	1		3					6
Phylum Platyhelminthes																			
Species 1					1					1									2
Phylum Nemertinea																			
Species 1			1			3													4

Nemerteans, which are hard to distinguish but were represented by only six individuals.

The patterns of species numbers and abundance of the benthic infauna are summarized in Tables 5 to 7. As seen in Table 5, there were more than twice as many species of polychaete than any other major group of benthic infauna. If the molluscs are considered as a group, there were more mollusc species than crustacean species. The crustaceans were the most abundant group, mostly due to the large numbers of Eudorella pacifica at Transect 5.

The total number of species at Transects 1 and 2 in April (Table 6) was exceptionally low because the samples were screened in freshwater, which caused many of the organisms to fragment excessively rendering them unidentifiable. Therefore, the benthic infauna data for these two transects in April cannot be compared with the data from the other transects and dates. All other samples were screened in ambient seawater.

The mean number of species, mean total abundance, and mean abundance of the four most abundant species (Nephtys corunata, Capitella capitata, Eudorella pacifica, and Nassarius mendicus) at Transects 1, 3, and 5 were each compared statistically between transects and dates using analysis of variance in conjunction with the Newman-Keuls multiple range test ($\alpha = 0.05$). The greatest number of species was found at Transect 1 in June and August (Table 6), but the number of species declined in December (not significantly). There was also no significant difference in numbers of species between April and December at Transect 5. The number of species at Transect 3 declined

Table 5. Distribution of the benthic infauna species collected in 1974 among major taxonomic groups.

Taxonomic group	Total species	Total abundance
Polychaetes	46	6050
Crustaceans	18	8929
Pelecypods	15	603
Gastropods	7	1042
Echinoderms	2	162
Coelenterates	1	6
Platyhelminthes	1	2
Nemerteans	1	4

Table 6. Total number of benthic infauna species at Transects 1-5 in 1974 (species/0.095 m^2)

	Transect				
Month	1	2	3	4	5
April	22	18	41	38	38
June	45	41	39	40	35
August	45	33	28	36	39
December	33		16		35

Table 7. Total abundance of benthic infauna at Transects 1 to 5 in 1974 (abundance/0.095 m^2)

	Transects				
Month	1	2	3	4	5
April	761	468	454	516	2686
June	966	1035	787	648	1591
August	710	733	573	565	1778
December	410		989		1185

significantly in August and December. In April there was no significant difference between the number of species at Transects 1, 3, and 5, but in August and December, the number of species at Transect 3 was significantly lower than at Transects 1 and 5.

As seen in Table 7, the greatest total abundance of benthic infauna occurred consistently at Transect 5 from April through December, but the mean abundance declined significantly in June and December. At Transect 1, the mean abundance also declined significantly from August to December, but the mean abundance at Transect 3 increased significantly from April to June and from August to December. In April, there was no significant difference between the abundance at Transects 1, 3, and 5. The mean abundance was significantly lower at Transects 1 and 3 than at Transect 5 in August, and in December, the abundance was significantly lower at Transect 1 than at Transects 3 and 5.

The greatest abundance of the polychaete worm, Nephtys corunata, was found at Transect 5 in April (259/0.095 m^2 or 2726/m^2), but then the mean abundance of N. corunata declined significantly in June and December. At Transect 1, the abundance of N. corunata also declined significantly in June and December. The abundance of N. corunata at Transect 3 was significantly lower than at Transects 1 and 5 from April through December. There was also a significant difference between the N. corunata abundance at Transects 1 and 5 from June through December. As seen in Table 6, there was a consistent, significant increase in the abundance of the polychaete worm, Capitella capitata, at Transect 3 from April to December, and also an obvious increase in its abundance at Transect 4. No C. capitata were

found at Transect 5, only one at Transect 2, and two at Transect 1.

The greatest abundance of the cumacean, Eudorella pacifica, was found at Transect 5 from April (2117/0.095 m^2 or 22,284/m^2) through December, but there was a significant decline in abundance of E. pacifica at this transect between April and June. Its abundance at Transect 1 was significantly lower than that at Transect 5 on all sampling dates, and decreased significantly between June and August and between August and December. At Transect 3, its abundance was significantly lower than at either Transect 1 or 5 on all sampling dates, and declined to zero in August and December.

There was too much within-sample variation to statistically evaluate the variation in abundance of the gastropod, Nassarius mendicus, between transects and dates but it can be seen in Table 4 that its abundance remained fairly constant at Transects 1, 2, and 5 on all sampling dates. At Transect 3, its abundance remained similar to that at Transects 1 and 2 from April through August, then dropped to the lowest abundance of N. mendicus observed (4/0.095 m^2 or 42/m^2) in December. Note the increase in its abundance at Transect 4 from April to the highest overall abundance observed (91/0.095 m^2 or 958/m^2) in August.

Two species diversity indices were calculated using the 1974 benthic infauna data and are summarized in Tables 8 and 9. The indices calculated at Transects 1 and 2 in April cannot be compared with the other indices because of the screening error described earlier. The mean Shannon-Weaver and Gleason indices, with their standard deviations, are plotted in Figs. 32 and 33, and were also analyzed with analysis of

Table 8. Mean H $\pm$ standard deviation at Transects 1 to 5 in 1974 (mean of five samples)

Month	Transect 1	2	3	4	5
April	1.22 $\pm$ 0.19	1.54 $\pm$ 0.10	1.92 $\pm$ 0.18	2.24 $\pm$ 0.25	0.94 $\pm$ 0.06
June	1.70 $\pm$ 0.31	1.33 $\pm$ 0.47	1.80 $\pm$ 0.48	2.01 $\pm$ 0.32	1.27 $\pm$ 0.06
August	1.89 $\pm$ 0.12	1.68 $\pm$ 0.34	1.37 $\pm$ 0.62	1.82 $\pm$ 0.60	1.51 $\pm$ 0.11
December	2.20 $\pm$ 0.21		0.74 $\pm$ 0.45		1.53 $\pm$ 0.28

Table 9. Mean d $\pm$ standard deviation at Transects 1 to 5 from 1974 (mean of five samples)

Month	Transects 1	2	3	4	5
April	1.76 $\pm$ 0.16	1.96 $\pm$ 0.20	3.90 $\pm$ 0.61	3.98 $\pm$ 0.50	3.14 $\pm$ 0.23
June	3.97 $\pm$ 0.48	3.62 $\pm$ 0.76	2.55 $\pm$ 0.78	3.92 $\pm$ 0.94	3.14 $\pm$ 0.50
August	3.97 $\pm$ 0.29	2.97 $\pm$ 0.86	2.68 $\pm$ 0.74	3.46 $\pm$ 1.06	3.88 $\pm$ 0.29
December	3.73 $\pm$ 0.55		1.31 $\pm$ 0.26		3.59 $\pm$ 0.60

(SQUARE=TRANSECT 1; OCTAGON=TRANSECT 3; TRIANGLE=TRANSECT 5)
(LINES=ST. DEV. OF TRANSECT 1; DASH=ST. DEV. OF TRANS. 3 AND 5)

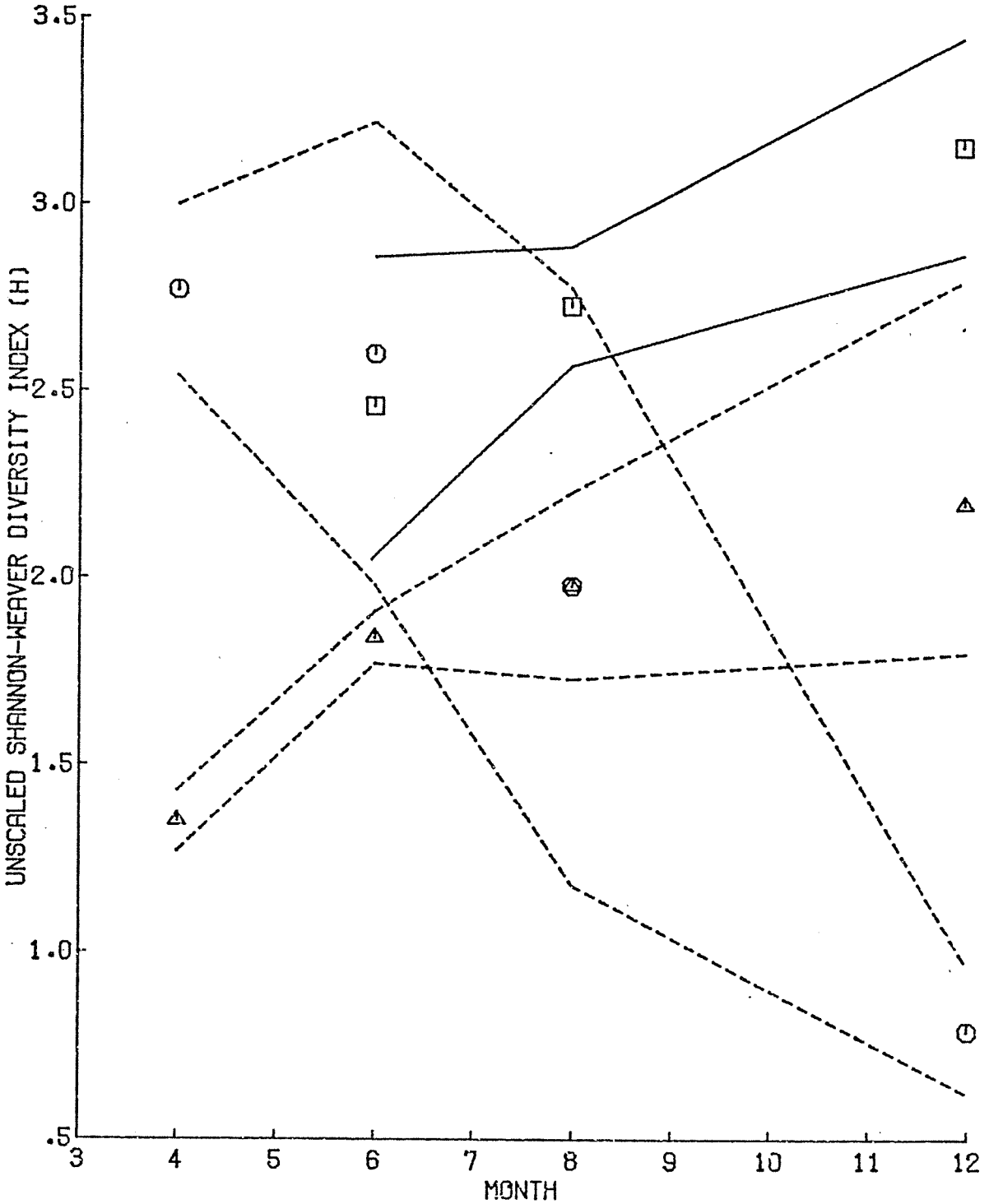


FIG. 32. MEAN UNSCALED SHANNON-WEAVER DIVERSITY (H) AND STANDARD DEVIATIONS AT TRANSECTS 1, 3, AND 5 FROM APRIL TO DECEMBER 1974.

(SQUARE=TRANSECT 1; OCTAGON=TRANSECT 3; TRIANGLE=TRANSECT 5)
(LINES=ST. DEV. OF TRANSECT 1; DASH=ST. DEV. OF TRANS. 3 AND 5)

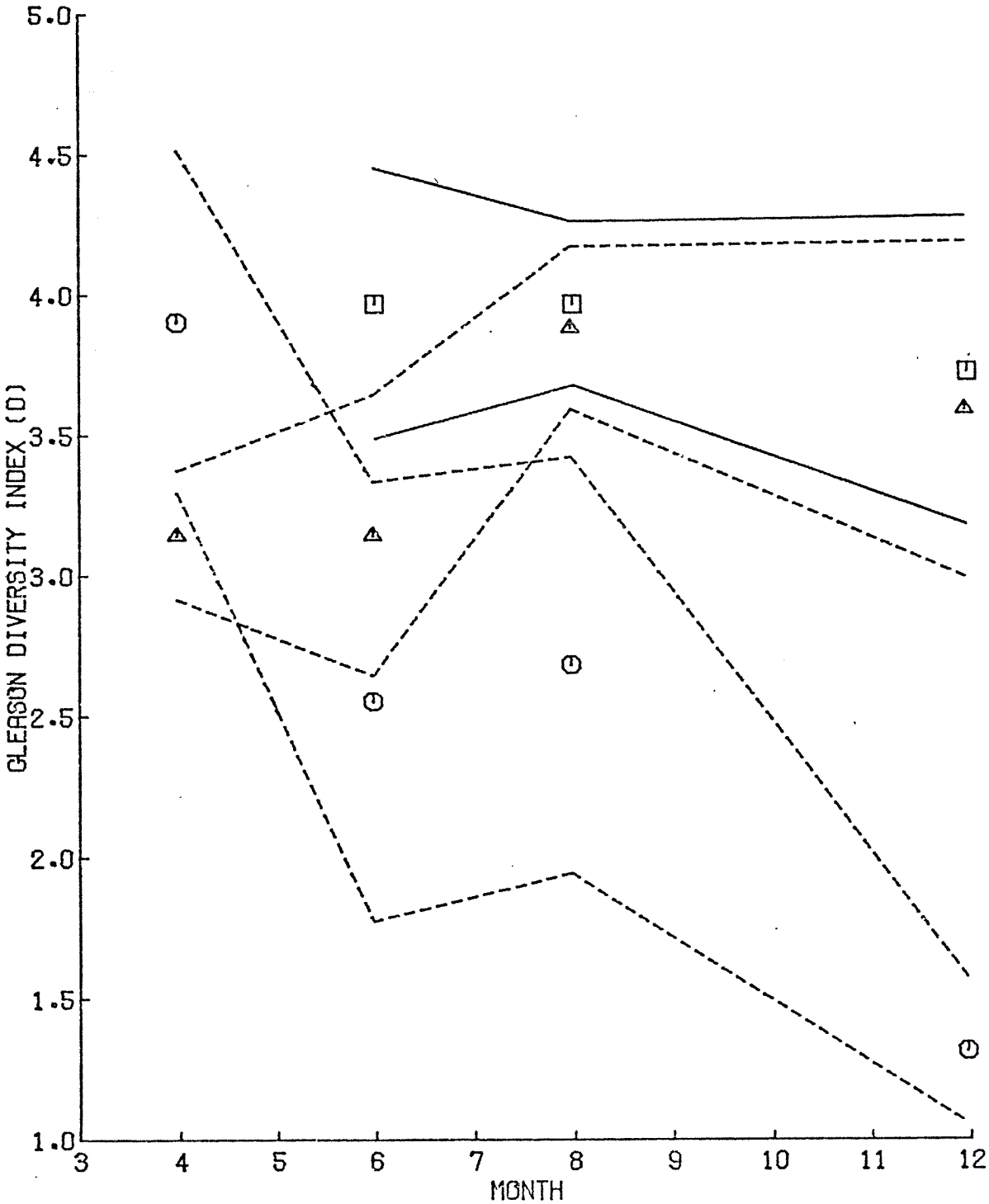


FIG. 33 . MEAN GLEASON DIVERSITY (D) AND STANDARD DEVIATIONS AT TRANSECTS 1, 3, AND 5 FROM APRIL TO DECEMBER 1974.

variance and the Newman-Keuls multiple range test ($\alpha = 0.05$) to test for significant differences between transects and sampling dates.

There was a significant increase in the Shannon-Weaver diversity index at Transect 5 between April and December; at Transect 3, it declined significantly between August and December; and in April, it was significantly lower at Transect 5 than at Transect 3. There was no significant difference between the three transects in June and August, but in December at Transect 3, it was significantly lower than at either control transect.

The diversity as measured by Gleason's index (Fig. 33) did not change significantly at either control transect from April to December but did decline significantly from April to June and from August to December at Transect 3. In both August and December, it was significantly lower at Transect 3 than at either control transect.

DISCUSSION

Water Quality

The major fluctuations in the water quality parameters at Henderson Inlet in 1974 were apparently related to the phytoplankton activity. Phytoplankton abundance was assumed to be directly proportional to the chlorophyll a concentration. As seen in Fig. 6, there were three major phytoplankton blooms in 1974, probably initiated by temperature, salinity, light, and nutrient conditions. The close relationship of chlorophyll a with salinity at the surface is seen in Fig. 4A where the three peaks in salinity coincide with the chlorophyll a peaks in Fig. 6A.

Soluble nitrate and ortho-phosphate are generally considered to

be the primary nutrients required for phytoplankton growth. Ryther and Dunstan (1971) demonstrated that marine phytoplankton growth in coastal waters is typically limited by soluble nitrate concentration, since there is generally an excess of ortho-phosphate in coastal marine waters. As seen in Fig. 18A, the phytoplankton at Henderson Inlet almost completely depleted the nitrate concentration at all stations near the surface and at the control station near the bottom in July. Note the correlation of this depression with the July peaks in chlorophyll a in Fig. 6 and the subsequent decrease in chlorophyll a in August, which is consistent with the increase in nitrate in August. The phosphate concentration was low in July at all stations, but did not decline below 25 ug/l (Fig. 21), and the minimum phosphate concentration at all stations occurred in May, which does not coincide with a peak in chlorophyll a. So it appears that nitrate is or can be a key limiting nutrient to phytoplankton growth in Henderson Inlet.

As seen in Figs. 6 and 7, the chlorophyll a concentrations near the surface in the mariculture slip (Stations 4A to 7A) were generally higher than those in the surface waters outside the mariculture slip (Stations 1A to 3A) June through September. This observation is verified in Table 10, where the mean surface chlorophyll a concentration at Stations 4 to 7 was consistently greater than the mean surface chlorophyll a concentration at Stations 1 to 3. A very intense phytoplankton bloom occurred in the mariculture slip August 29 through September 12, and an increased mortality of salmon in the pens also occurred during the same period, indicating that an abundance of algal cells can possibly be related to salmon mortality.

Table 10. Comparison of the mean surface chlorophyll a concentration at Stations 1 to 3 with the mean surface chlorophyll a concentration at Stations 4 to 7, March through December 1974.

Dates	Stations 1 to 3	Stations 4 to 7
March 3	1.3	1.0
April 24	6.0	4.2
May 26	1.9	2.0
June 26	11.0	15.0
July 29	12.8	20.1
Aug 27	10.3	19.5
Sept 29	17.1	46.5
Oct 29	7.9	6.3
Nov 25	2.2	2.3
Dec 28	0.7	0.5

The tidal current in Henderson Inlet is very weak. A flowmeter (Savonius rotor type) was used to measure the tidal current at the water sampling stations several times during the summer and fall of 1974. Even during maximum ebb and floodtides, there was usually no measurable current and the strongest current measured was only 0.1 knot. Decreased mixing and increased vertical stability of the water column in Henderson Inlet from June through September is demonstrated in Table 11 by the increased difference between mean surface and mean bottom temperatures. The greater temperature differentials at Stations 4 to 7 in Table 11 illustrate that the water column in the mariculture slip was even more stable than the water column outside the mariculture slip (Stations 1 to 3). A physical barrier to mixing within the mariculture slip is provided by the surrounding log rafts and the netting of the salmon pens (Moring, 1973).

Welch (1967) demonstrated that increased stability of the water column is a primary hydrographic factor stimulating phytoplankton activity. The increased phytoplankton density in the mariculture slip probably results from the fact that the phytoplankton are retained in the mariculture slip for a relatively long period of time and exposed to slightly increased concentrations of nutrients excreted by the fish.

A rough estimate of the amount of nutrients available from the mariculture fish can be arrived at using the data collected by Liao and Mayo (1970) from freshwater salmonid fish hatchery effluents. They found average values of 0.166 lbs of ammonia, 0.401 lbs of nitrate, and 0.033 lbs of phosphate/day/100 lbs of fish in the numerous salmonid hatchery effluents that they studied. Applying these values to the

Table 11. Comparison of the mean temperature (C) difference between surface and bottom at Stations 1 to 3 with the mean temperature difference at Stations 4 to 7, March through December 1974.

Dates	Stations 1 to 3	Stations 4 to 7
March 3	0.4	0.4
April 24	0.4	0.4
May 26	1.1	1.8
June 26	0.4	0.3
July 29	1.8	2.9
Aug 27	1.7	2.3
Sept 29	0.4	0.9
Oct 29	0.3	0.0
Nov 25	0.2	0.1
Dec 28	0.7	0.5

fish biomass in the high- and low-density floats in July, we find that the fish were producing approx 10.0 lbs of ammonia/day, 24.0 lbs of nitrate/day, and 2.0 lbs of phosphate/day. If these nutrients were retained within the volume of the fish pens in this example, 5 mg ammonia/l, 11 mg nitrate/l, and 1 mg phosphate/l would result. The actual amount of nutrients produced by the fish at Henderson Inlet was probably quite different from these calculated values, due to the effects of salinity and temperature on the metabolism of the fish and the efficiency of feeding. However, these calculations point out the fact that the fish were continuously producing a significant amount of nutrients, to which the phytoplankton were exposed in the mariculture slip.

Eppley, et al (1972) showed that marine phytoplankton take up nutrients very rapidly as the nutrients leave a coastal sewer outfall. They used this argument of rapid uptake to explain the fact that nitrate and phosphate concentrations at the mouth of the sewer outfall were similar to concentrations at control stations when there was a phytoplankton bloom near the outfall. This same phenomenon was observed with the nitrate concentrations in the surface waters of Henderson Inlet from June through September. The nitrate concentration in the surface waters at the outer control and the mariculture slip were very similar in June, July, and September, but the concentration at the outer control increased to twice that inside the mariculture slip in August. There apparently was a die-off of phytoplankton in Henderson Inlet in August, but relatively fewer algal cells apparently died inside the mariculture slip than at the outer control.

One might expect the ammonia, nitrate, and phosphate concentrations in the surface waters of the mariculture slip to increase above the

concentrations at the outer control after the final phytoplankton die-off in October, based on the earlier calculations and the increase in fish biomass observed in Fig. 26. Destratification of the water column (as seen in Table 12 by the decrease in temperature differential between the surface and bottom in October) is one of the main reasons for the phytoplankton die-off in Henderson each fall. This destratification generally increases the mixing throughout Henderson Inlet. This increased mixing is apparently adequate to carry the soluble nutrients (ammonia, nitrate, and phosphate) away from the mariculture slip in the fall and winter.

The DO and ammonia concentrations near the bottom were obviously affected by the phytoplankton activity, as seen by the coincidence of peaks in July and September in Fig. 6B, with the peaks in Fig. 12B, and with the depressions in Fig. 16B. The DO is directly correlated with chlorophyll a since it is produced by the phytoplankton during the daylight hours, and ammonia concentration is inversely correlated with chlorophyll a, since it is a source of nitrogen for phytoplankton (Eppley, et al, 1971; Ryther and Dunstan, 1971).

In the surface waters, it appears that this relationship between DO, ammonia concentration, and phytoplankton activity held only at the outer control station (Station 1A). The DO at the surface stations inside the mariculture slip (Stations 4A to 7A) declined in July, while the ammonia concentration at these stations increased (212 ug/l at Station 5A). This decline in DO and increase in ammonia within the surface waters of the mariculture slip in July probably results from the respiration and excretion of the salmon in the pens during a period

Table 12. Water quality data collected from Henderson Inlet, 1957-1958 (Collias, Dermody, and Barnes, 1962)

	Dec.	April	May	June	July	August	Sept.	Oct.
Surface temperature (C)	8.7	10.4	14.3	16.2	15.9	16.2	17.6	13.9
Bottom temperature (C)	9.1	9.5	11.8	12.8	14.8	15.4	15.2	13.9
Surface salinity (ppt)	27.4	27.2	27.7	28.0	28.9	29.2	28.8	29.6
Bottom salinity (ppt)	29.4	28.4	28.5	28.7	29.2	29.3	29.6	29.8
Surface dissolved oxygen (ppm)	7.8	9.7	10.8	8.8	6.9	8.2	9.7	7.1
Bottom dissolved oxygen (ppm)	7.1	7.9	9.6	8.1	7.3	7.4	6.4	6.7
Surface ortho-phosphate (ppb as PO_4)	80.4	50.7	42.8	51.9	46.1	63.1	75.9	67.6
Bottom ortho-phosphate (ppb as PO_4)	83.0	64.6	62.4	46.7	66.7	64.6	74.3	70.0

of limited mixing (Table 11). The relatively high phaeophytin concentrations in the surface waters of the mariculture slip in July indicate that there was a large number of dead algal cells present in the mariculture slip. Decomposition of these dead algal cells could also contribute to the low DO and high ammonia levels (Welch, 1967). Note that the September peak in phaeophytin at the outer control (Fig. 9B) also corresponds with a lower DO (Fig. 12B) and higher ammonia concentration (Fig. 16B) than observed within the mariculture slip.

Water samples were collected only during the morning low tide, because it is assumed that the daily minimum DO and maximum ammonia and phaeophytin occurs at this time. The 24-hr water sampling data collected on September 15 and 16 demonstrate that this assumption is probably valid (Figs. 11, 14, and 18). These conditions are probably caused by the death and decomposition of phytoplankton in the shallows at the head of Henderson Inlet at high tide. The decomposing phytoplankton lower the DO and increase the nutrient, ammonia, and phaeophytin concentrations of these waters, which are carried out to the mariculture slip area by the outgoing tide. Also, the phytoplankton have been respiring all night.

The minimum DO observed in Henderson Inlet during 1974 was 5.6 mg/l at Station 6A in October. The Washington State Department of Ecology has classified the waters of Henderson Inlet as Class AA, and indicates that the DO in these waters should exceed 7.0 mg/l (State of Washington, 1973). However, DO's lower than 7.0 mg/l were measured at all stations, including the outer control, in Henderson Inlet in October. Collias,

Dermody, and Barnes (1962) also measured DO's lower than 7.0 mg/l in Henderson Inlet in July, September, and October of 1957 and 1958 (Table 12). Low DO's in many parts of Puget Sound during late summer and early fall are caused by an influx of dense, more saline, cool water from the Strait of Juan de Fuca (Barnes and Collias, 1958). Since the DO in Henderson Inlet seasonally drops below 7.0 mg/l, a standard of 7.0 mg/l is obviously too high. The Committee on Water Quality Criteria (1972) points out that laboratory and field observations have shown that DO values lower than 4 to 5 mg/l as daily minimum values for periods of several days are known to cause deleterious effects on marine organisms. Therefore, they recommend that marine DO's be maintained above 4 mg/l at all times. This appears to be a more reasonable criterion to apply to Henderson Inlet water quality. Based on this criterion, it appears that the DO in Henderson Inlet waters was not adversely affected by the mariculture facility.

The highest ammonia concentration measured at Henderson Inlet in 1974 was 212 ug/l as NH_3 at Station 5A in July. The toxicity of ammonia is known to be due to the concentration of undissociated ammonia in solution, which is dependent on the pH, temperature, and salinity of the water. The pH was measured at the seven water sampling stations in Henderson Inlet from March through July and found to be 7.75 at Station 5A in July. Using the water temperature, pH, and salinity measured at Station 5A in July, it is estimated that the concentration of undissociated ammonia was approx 10 µg/l as N. The Committee on Water Quality Criteria (1972) has suggested that concentrations of undissociated ammonia equal to or exceeding 400 µg/l as N constitute a

hazard to marine biota, and levels less than 10 µg/l as N present minimal risk of deleterious effects. Based on this criteria, it appears that the mariculture facility did not release dangerous amounts of ammonia into the waters of Henderson Inlet.

The major effect of the mariculture facility on the water quality of Henderson Inlet in 1974 was a stimulation of phytoplankton growth, but the effects were apparently limited to the surface waters inside the mariculture slip. Also, note that the highest chlorophyll a concentration was found at the outer control in July, indicating that intense phytoplankton blooms are a natural occurrence in Henderson Inlet. Domenowske and Matsude (1969) indicate that phytoplankton blooms producing chlorophyll a readings of 200 µg/l are not unusual in Puget Sound.

Benthic Environment

The mariculture operation also introduces a certain amount of insoluble, particulate matter into the waters of Henderson Inlet, mostly in the form of fish feces, excess fish food, and fouling organisms washed off of the nets. As indicated earlier, the tidal flow in Henderson Inlet, and particularly in the mariculture slip, is very limited. Since the waters of the mariculture slip are only 30 ft deep, insoluble particulate matter probably falls to the bottom directly under the fish pens.

As a rough estimate of the amount of settleable solids produced by the mariculture facility, measurements from freshwater salmon hatchery effluents can be used. The U. S. Bureau of Sport Fisheries and Wildlife (Anonymous, 1974) has calculated that approx one-third of

of the dry weight of the food fed to salmon in a hatchery will result in settleable solids in the effluent. Of course, this value is very dependent upon the feeding efficiency of the operation and the metabolism of the fish, but it probably approximates the potential amount of material that will accumulate under the pens. Using this approximation on the cumulative dry weight of food (dry weight of OMP is approx 70% of the wet weight) introduced into the high-density pens, it appears that approx 2 tons of organic matter probably settled to the bottom under the high-density pens by December of 1974.

The fish food used at Henderson Inlet (OMP) is 7.7% nitrogen by weight and 47% carbon by weight, with a carbon/nitrogen ratio of 6/1. Stephens, Sheldon, and Parsons (1967) found that the majority of the organic matter accumulating on the bottom of Departure Bay, British Columbia, from May to July originated from the phytoplankton in the water column. During this period, the carbon/nitrogen ratio of the settleable solids was approx 6/1, which is the average ratio they found in living phytoplankton and is the same ratio found in the fish food at Henderson Inlet. Stephens, Sheldon, and Parsons (1967) found that there were two yearly peaks in sedimentation rate, one due to phytoplankton (May to July) and another due to an influx of terrigenous matter (October to December), which had a higher total weight and carbon/nitrogen ratio (10/1). The general seasonal cycle of sedimentation in Henderson Inlet is probably similar to the cycle observed by Stephens, Sheldon, and Parsons, except that there is possibly an additional input from the log rafts surrounding the mariculture site. Therefore, one would expect the organic input from the salmon pens to increase the supply of

both carbon and nitrogen during the entire growing season, but the input of nitrogen would be especially significant in the fall and winter.

The carbon and nitrogen concentration of the surface sediments is a measure of the composition of accumulating organic matter, as well as how the accumulating organic matter is interacting with the benthic community. The carbon and nitrogen concentration of the sediments at all transects in the mariculture slip (1 to 4) was significantly higher than the concentration at the outer control, probably because organic matter such as bark and wood fibers from the log rafts had accumulated in the sediments of the log-rafting area. There is visibly more bark and wood fiber in the sediments of the mariculture slip than in the sediments of the outer control, which are mostly silt. The grain-size distribution of the sediments at all transects will be analyzed to quantify the description of the sediments.

There was a logarithmic increase in carbon concentration of the sediments under the high-density pens from April to December, while no increase was observed at either control transect (Fig. 28). This increase most likely resulted from an accumulation of excess food and feces under the pens, as well as an increase in biomass of bacteria and meiofauna. A similar increase was observed under the low-density pens. The concentration of nitrogen in the sediments under the high-density pen increased rapidly from April to June, and then more slowly between June and December, apparently approaching some asymptotic value. One explanation for this decline in the rate of accumulation of nitrogen is that a species, or group of species, of benthic bacteria was converting the organic nitrogen to soluble nitrate or ammonium and

releasing it into the water column. Possibly the nitrogen passed through the food web to an organism such as Cancer gracilis, which then emigrated from the area. Regardless of the mechanism, it can be seen that nitrogen is much more dynamic than carbon in the sediments of Henderson Inlet.

The appearance of Beggiatoa, the white, sulfur bacteria, under the pens in July indicates that there was also a supply of sulfur in the organic matter accumulating under the pens, which is reduced to hydrogen sulfide by anaerobic bacteria, such as Desulfovibrio, then oxidized to sulfate by Beggiatoa and released to the water column. Therefore, the presence of Beggiatoa indicates the presence of hydrogen sulfide (a very toxic substance).

Table 4 shows that there was a substantial increase in the abundance of C. capitata under the high-density pens from April to December. Using Reish's (1960) classification of indicator organisms, the increase of C. capitata associated with an increase in the carbon content of the sediments indicates that there was an accumulation of organic matter under the pens (polluted zone), which resulted in a major change in the structure of the benthic community.

Two other polychaete species, Gyptis brevipalpa and Dorvillea rudolphi, increased in abundance under the high-density pens along with C. capitata, indicating that they are also transgressive species. C. gracilis was also noticeably transgressive. C. gracilis possibly eats C. capitata and was attracted by the abundance of this species under the pens. N. corunata and E. pacifica are obviously regressive species, since E. pacifica disappeared from Transect 3 in August and N. corunata disappeared by December. The molluscs, N. mendicus,

Macoma, and Mysella tumida are apparently indifferent species, occurring consistently at Transect 3 from August through December.

The species composition of the benthos at the outer control was noticeably different from the species composition of the benthos within the mariculture slip. E. pacifica, Pinnixa schmitti, Amphiodia urtica, Psephidia ovalis, and the amphipods were consistently more abundant at the outer control than at any other transect, while N. mendicus and Odostomia tacomaensis were consistently less abundant. Stylatula elongata, polychaete species 1 and 15, and amphipod species 3 were found only at the outer control. The difference in species composition is probably related to the difference in carbon-nitrogen composition and particle size distribution of the sediments at the outer control compared to the sediments within the mariculture slip. Both of these factors are related to the amount of bark and wood fiber in the sediments.

Both diversity indices decreased significantly and consistently from April to December under the salmon pens, while they remained fairly constant or increased at all other transects. This drop in diversity results from the significant decrease in the number of species while the total abundance remained fairly constant. Most of the total abundance at Transect 3 in December was due to one species (C. capitata) Both diversity components were adversely affected, indicating that there was an accumulation of organic matter under the salmon pens which imposed a significant stress on the benthic community.

There was also a seasonal change in the Shannon-Weaver diversity index, which was observed at all control transects. This seasonal change at the controls is probably caused by the significant decline

in total abundance observed at Transects 1 and 5, while the number of species remained fairly constant. The decrease in abundance of the more abundant species results in greater evenness, as well as greater species richness. It appears that the Shannon-Weaver index is the most sensitive index to changes in benthic community structure at Henderson Inlet, since Gleason's index did not change significantly at the control transects.

In attempting to relate the changes in benthic diversity observed under the high density pens to the physical environment, it was noticed that the unscaled Shannon-Weaver diversity index declined logarithmically from April to December while the carbon concentration of the sediments increased logarithmically over the same period. Regression analysis shows a very good linear fit (Fig. 34) between carbon concentration and the Shannon-Weaver index at Transect 3. This demonstrates a definite relationship between the carbon concentration (an index of accumulation of organic matter) and species diversity (an index of community health). A similar relationship is observed (Fig. 35) between the carbon concentration in the sediments at Transect 3 and the biomass of fish in the overlying high-density pens, indicating that carbon concentration is indeed an index of the accumulation of settleable solids under the salmon pens. Fig. 36 demonstrates that there is a definite linear relationship between the fish biomass in the high-density pens and the health of the underlying benthic community.

A more extensive regression analysis will be developed in the Final Report in an attempt to relate the spatial and temporal variation in the unscaled Shannon-Weaver diversity index to spatial and temporal

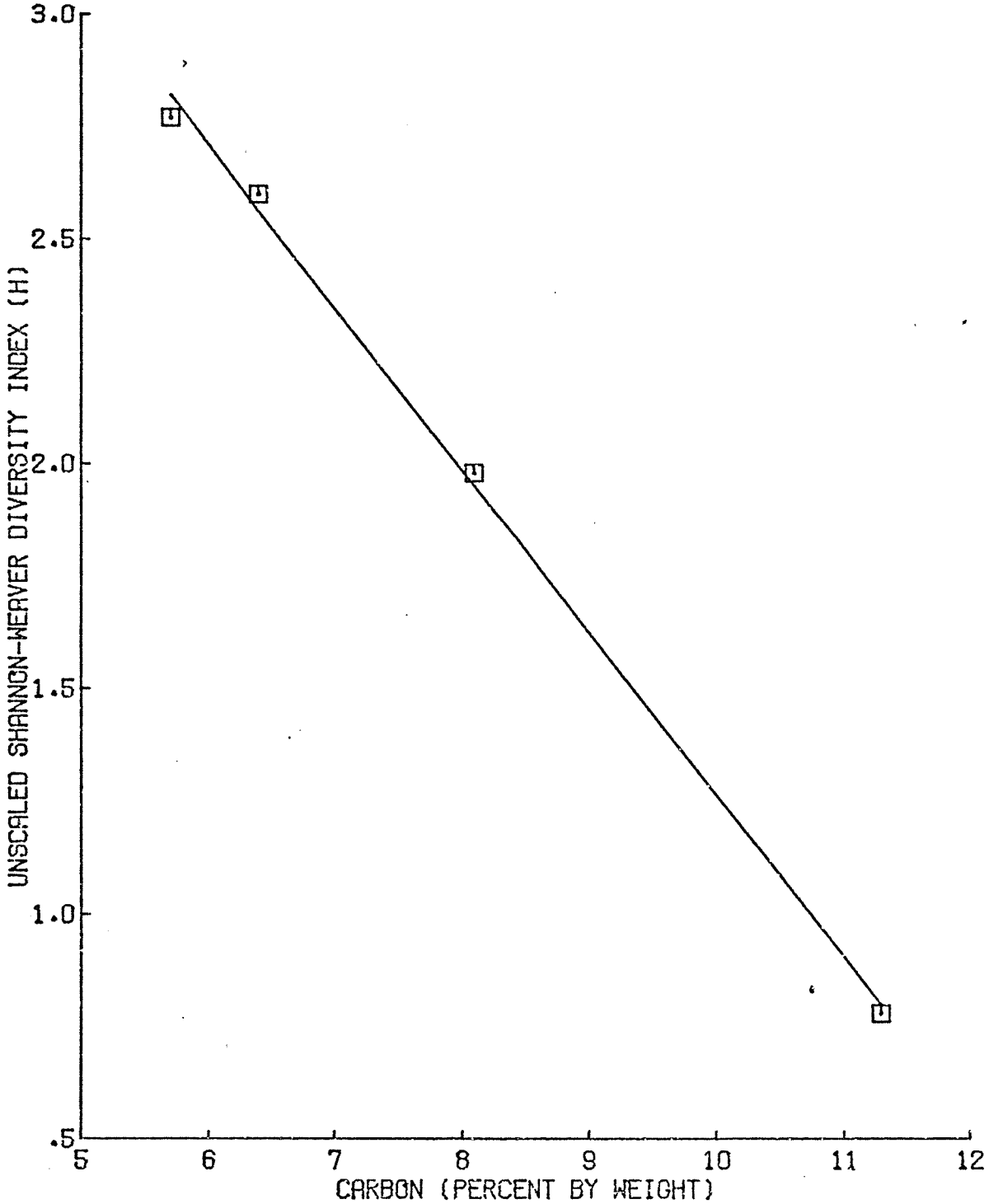


FIG. 34. REGRESSION OF CARBON CONCENTRATION VERSUS THE SHANNON-WEAVER DIVERSITY INDEX (H) AT TRANSECT 3 IN 1974.

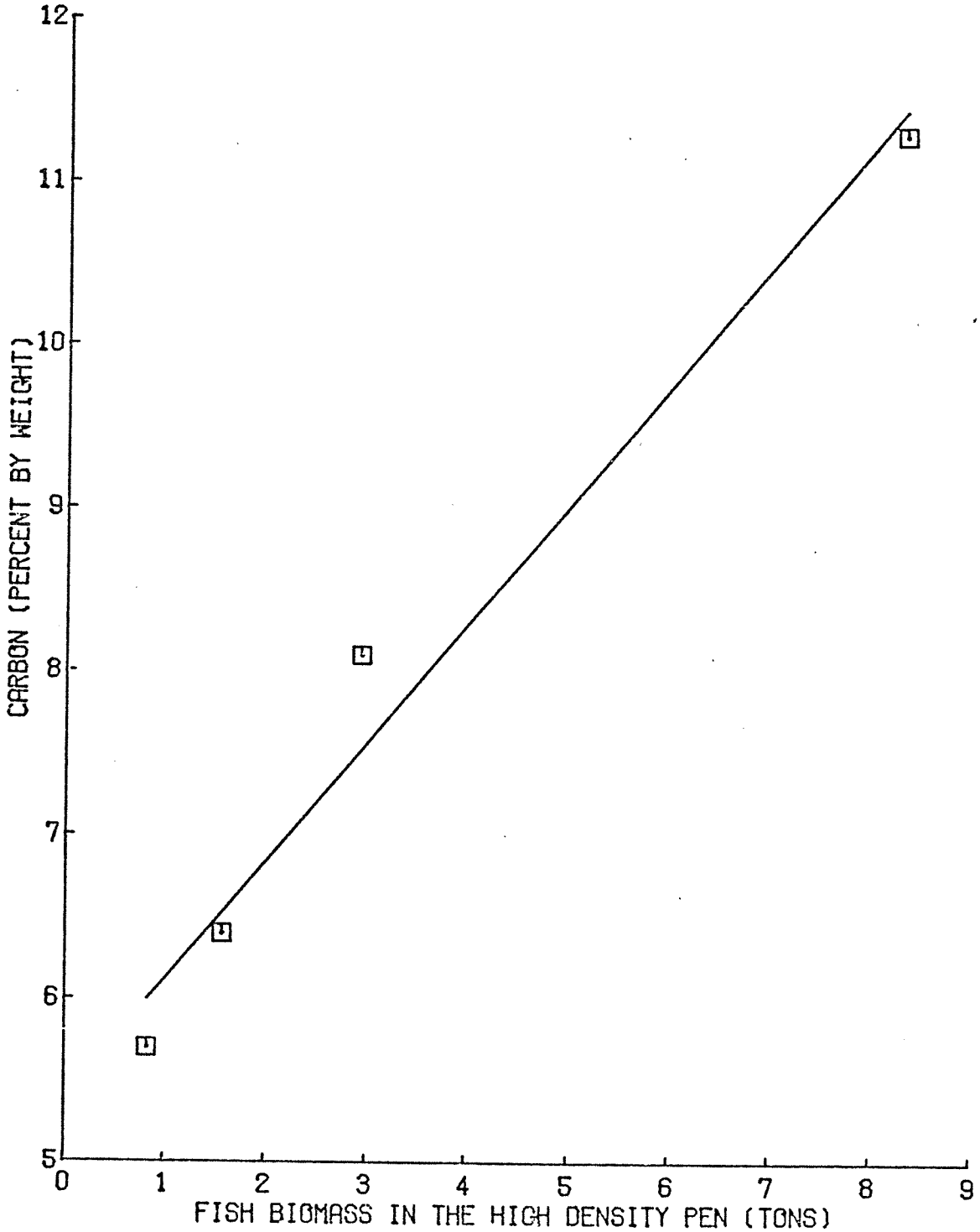


FIG. 35. REGRESSION OF THE HIGH DENSITY PEN FISH BIOMASS VERSUS CARBON CONCENTRATION AT TRANSECT 3 IN 1974.

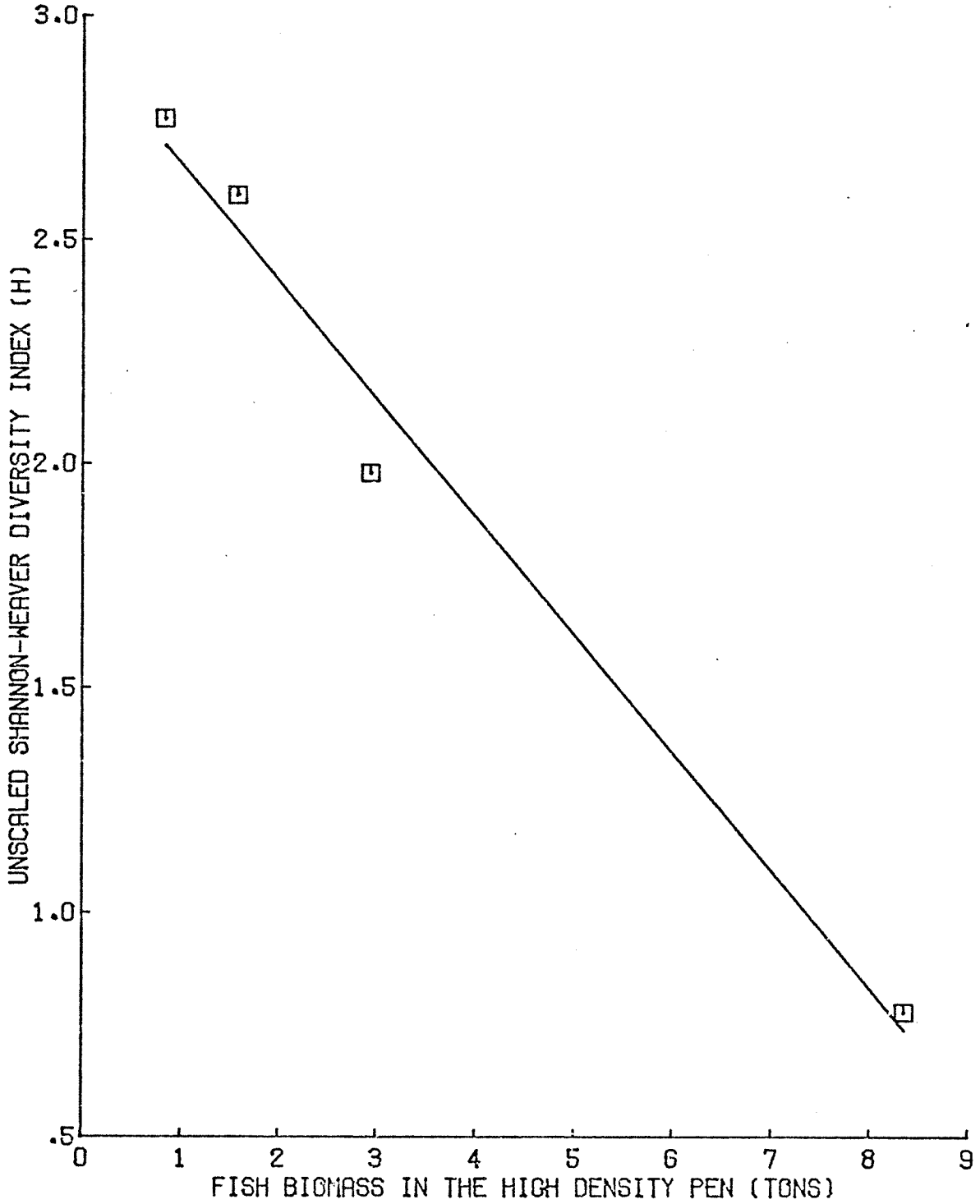


FIG. 36. REGRESSION OF THE HIGH DENSITY PEN FISH BIOMASS VERSUS THE SHANNON-WEAVER DIVERSITY INDEX (H) AT TRANSECT 3 IN 1974.

variations in carbon and nitrogen concentration and particle size distribution of the sediments, sedimentation rate, benthic oxygen consumption, and temperature and salinity of the overlying water. Biological factors such as predation, recruitment, and deposit feeder-suspension feeder relationships will be discussed.

SUMMARY AND CONCLUSIONS

1. The major fluctuations in water quality at Henderson Inlet in 1974 were related to phytoplankton activity.

2. The net pens and surrounding log rafts apparently create a barrier to surface mixing when combined with the seasonal stratification of the water column in the summer. This decreased mixing stimulated phytoplankton growth in the surface waters of the mariculture slip from June to September as a result of increased availability of nutrients from the fish metabolism and fish food.

3. There is apparently adequate mixing of the water column in spring, fall, and winter to prevent the accumulation of nutrients in the mariculture slip.

4. There is apparently adequate mixing at all times to prevent a significant decline in dissolved oxygen or accumulation of ammonia in the mariculture slip as a result of fish respiration and excretion, even though there is a seasonal decline in dissolved oxygen concentration in Henderson Inlet.

5. The initial sedimentation rate studies and surface sediment analysis indicate that there is an accumulation of organic material, rich in carbon and nitrogen, under the salmon pens.

6. The significant increase in abundance of a well-known transgressive indicator species, Capitella capitata, indicates that the benthic community under the salmon pens was significantly affected by the accumulation of organic material.

7. The significant decrease in two species diversity indices also indicates that the benthic community was signficantly affected.

8. The inverse linear relationship between the unscaled Shannon-Weaver diversity index of the benthic community under the high-density pens and both the biomass of fish in these pens and carbon concentration of the sediments under them indicates that the effects on the benthic community were related to the mariculture facility, through the accumulation of organic matter under the salmon pens.

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PART III

A COMPARISON OF GROWTH AND SURVIVAL OF CULTURED SPOT PRAWNS *PANDALUS PLATYCEROS* BRANDT AT TWO SALMON FARMING SITES IN PUGET SOUND

by

John Eric Rensel

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ACKNOWLEDGEMENTS

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INTRODUCTION

In the past five years several commercial salmon culture projects utilizing marine floating pens have been developed in the Pacific Northwest. At the conception of this study an effort was made to identify commercially valuable species that would be compatible with salmon. By rearing several species together or in adjacent pens I thought the economic base of net-pen aquaculture could be expanded without large additional expenditures. The purpose of this study was to investigate the suitability of culturing a potential companion crop species, the spot prawn Pandalus platyceros (herein referred to as prawn).

Inherent characteristics that make this temperate water species a likely candidate for aquaculture are (1) its advanced stage of development at hatchout; (2) large size at maturity (4 to the pound); (3) adaptability to both vertical and horizontal substrates; (4) willingness to accept a variety of foods; (5) lack of overt canabalistic tendencies; (6) ability to copulate in net-pens; (7) high market price and consumer acceptability.

The objectives of this study were to (1) compare prawn growth at two sites, one with seasonally warmer waters that was conjectured to accellerate growth; (2) evaluate several methods of rearing prawns that could be incorporated into the existing pen reared salmon industry; (3) monitor environmental parameters and conditions at both sites and relate these to growth and survival or prawns.

REVIEW OF PERTINENT LITERATURE

Although prawns occur along the west coast of North America from Unalaska to San Diego (Rathbun, 1904), detailed studies of natural populations have centered on southern British Columbia. Berkeley (1930) surveyed larval development, sexual development, growth and migration for five of the most common pandalids in British Columbia, including the prawn. Butler (1964) gathered detailed information on juvenile and adults of nine pandalid species; their growth patterns, reproductive habits and ranges. His work serves as a standard for comparing growth of prawns cultured in this and other studies.

Groundwork for prawn culture was laid by Price (1969, Price and Chew, 1972) who successfully reared the species to post-larval stage. These works describe larval development and culture systems such as a hatching container. Basic environmental requirements of larvae and juveniles were established by Wickins (1972) who also evaluated the species aquaculture potential in various culture systems.

More extensive studies of aquacultural aspects of larval and juvenile prawns were described by Kelly et al. (1976, in press). The authors concluded that prawn culture by itself (monoculture) is not feasible due to restricted rates of growth. They do point out that polyculture systems, such as abalone and prawn combinations, may have some potential.

As a possible means of reducing production costs Prentice (1975) has been investigating the feasibility of net-pen culture for prawns in Puget Sound, Washington. Laboratory reared juveniles and wild trapped adults have been held in non-capital intensive net-pens similar to those

used by marine salmonid growers (Mahnken, 1975). Relatively slow growth rates are not forseen to be restrictive to commercial prawn ventures if mono- and polyculture techniques can be imposed and the commercial value continues to rise.

SITE DESCRIPTION

Clam Bay

The National Marine Fisheries Service's experimental aquaculture station on the west side of the Kitsap Peninsula near Manchester, Washington, was chosen as one of two culture sites (Fig. 1). Wet laboratories and floating pens are situated at the terminus of a pier 244 m (800 ft) in length. Depths near the pens range from 9 to 14 m, depending on the stage of the tidal cycle.

Tidal currents of 0.4 knots at maximum flood, 1.0 knots at maximum ebb, maintain relatively constant salinity values in this bay with extremes of 27 and 31 ppt and an average value of about 30 ppt. Water temperatures range from about 6° C in January to 15° C in August. In the 1972-73 salmon rearing season visibility (secchi disc values) ranged from 5.7 m in September to 7.5 m in November, while dissolved oxygen values ranged from 5.2 ppm in December to 8.4 ppm in July (Moring, 1973). Hydrological values such as these indicate extensive water exchange with the central basin of Puget Sound.

Prawns had been reared at the Clam Bay site in the previous year. Survival and growth at this site was quite good and it was felt that Clam Bay animals could act as a control for those reared at Henderson Inlet.

Henderson Inlet

Henderson Inlet (Fig. 1) located near the southern end of Puget Sound was selected as the other site for this study. It is the location of the Weyerhaeuser Company's South Bay log dumping and rafting facilities. In 1973, Weyerhaeuser initiated a pilot scale salmonid aquaculture project at the site. In 1974, the project was expanded to include the rearing of .25 million yearling coho and zero aged chinook salmon in a log raft storage slip at the outer edge of the facility.

Since Henderson Inlet is relatively shallow (10 m mid channel) and located in southern Puget Sound, seawater exchange with the northern sound and oceanic water masses is more restricted than the Clam Bay site. Water quality conditions are subject to greater variations in temperature and salinity due to the slow rate of flushing and the local effects of freshwater runoff and solar radiation. Hydrographic baseline data was collected from surface waters during the 1973 pilot salmon project (Snyder et al., 1974). Salinity values ranged from 27.5 ppt in December to 32 ppt in late summer but remained between 30 and 32 ppt from June until December. Mean water temperature at 2 m ranged from 6° C in January to 19° C in August and remained near 16° C in the summer months. Water visibility, as measured by secchi disc, ranged from 1.5 m in June to 5.9 m in September. Dissolved oxygen levels reached a maximum of 13.0 ppm in September and dropped to 4.5 ppm in October.

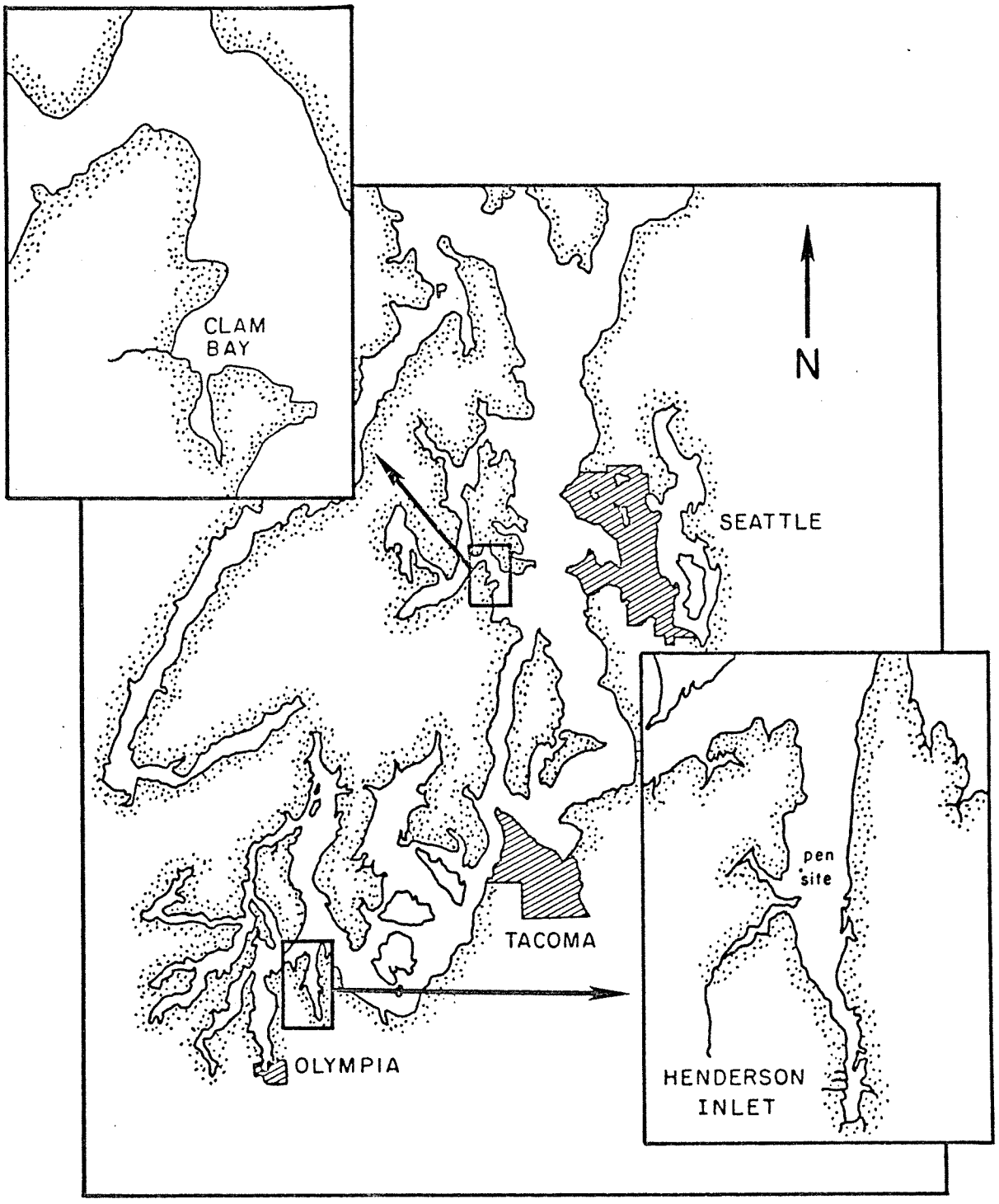

Fig. 1. Map of Puget Sound, Washington, showing enlarged views of Henderson Inlet and Clam Bay.

METHODS AND MATERIALS

Experimental Animals

Zero-aged laboratory reared prawns will be referred to as juveniles following the developmental scheme proposed by Williamson (1969) and used by Price (1969) for this species.

Laboratory reared juveniles were obtained from the National Marine Fisheries Service and stocked in net-pens when the mean carapace length reached 5 mm. Initial juvenile carapace lengths ranged from 2.7 to 11.1 mm.

Yearling prawns used in this study were obtained from commercial "pot" fishermen on Hood Canal in early May 1974. The catch of 2000+ prawns was transported by means of styrofoam coolers from the boats to a tank truck and onto the Clam Bay site for acclimation. A week later approximately half (1250) of the prawns were transported to the Henderson Inlet for two additional weeks of acclimation. Average carapace length was 25.8 mm and ranged from 19.5 to 29.0 mm. Average weight was 11.1 grams and ranged from 4.8 to 16.4 grams.

Zero aged chinook salmon (Oncorhynchus tshawytscha) for this study were obtained from the Washington Department of Fisheries, Hoodsport hatchery. The fish averaged 12 grams (.026 lb) and had a mean fork length of 99 mm (3.9 inches).

Enclosures

Yearling prawns were cultured in rectangular net-pens that measured 1.2 m x 1.8 m x 1.8 m deep (4 ft x 6 ft x 6 ft) the mesh size being 6.35 mm (1/4 inch) bar length. Polyvinyl chloride pipe frames were

placed in the pen bottoms to maintain the pens rectangular shape. Total immersed substrate available to the prawns was 11.5 m^2 (124 ft^2) per pen. Each pen was stocked with 112 prawns for an initial density of 9.7 prawns per m^2 (0.9/ft^2). Pens used to rear the juvenile prawns were similar in size but of a smaller mesh opening (4.8 mm or 3/8 inch bar) and had two panels sewn in vertically dividing each pen into three equal chambers. Total immersed substrate area was 6.3 m^2 (68 ft^2) per chamber. Each chamber was stocked with 45 prawns which meant an initial density of 7.1 prawns per m^2 (0.7/ft^2).

Benthic-cages at Henderson Inlet were constructed of vinyl coated wire mesh having an aperture size of 13 mm (1/2 inch) bar length. The cages measured 0.9 m x 0.9 m x 0.5 m deep (3 ft x 3 ft x 1.5 ft) with a useable substrate area of 2.5 m^2 (27 ft^2). Yearling prawns were stocked 21 to the cage for an initial numerical density of 8.4 prawns per m^2 (0.8/ft^2).

Treatments

Treatments duplicated at each site (Table 1, No. 1 and 2). Juvenile prawns at each site were fed raw mussle (Mytilus edulis) meat and fronds of the kelp Nereocystis leutkeana as a dietary supplement. Previous experimenters (Earl Prentice, NMFS, personal communication; Forster, 1970; Wickins, 1972) have found raw mussel to be a satisfactory feed for marine prawns.

Three replicate groups of yearling prawns at each site received chopped, frozen geoduck clam (Panope generosa) processing wastes. Japanese culturist routinely feed littleneck clams (Venerupis japonica)

Table 1. Treatment

No.	Location	Rearing System	Life Stage	Feed	No. of Replicates
1	Clam Bay & Henderson Inlet	surface net	juvenile	mussel & kelp	3
2	Clam Bay & Henderson Inlet	surface net	yearling	clam processing wastes	3
3	Clam Bay	surface net	yearling	non-supplemented	3
4	Henderson Inlet	surface net with salmon	yearling	uneaten OMP & fish feces	3
5	Henderson Inlet	surface net	yearling	OMP	3
6	Henderson Inlet	benthic cages under salmon pens	yearling	non-supplemented	4
7	Henderson Inlet	benthic cages in control areas	yearling	non-supplemented	4

and find it to be a satisfactory diet in terms of growth although very expensive (Bardach et al., 1972).

Treatments initiated at only one site (Table 1, No. 3 to 6). The following diet/culture systems were evaluated using yearling prawns at one or the other site.

(1) Clam Bay - non supplemented - surface pen reared.

This group received no direct feed and was allowed to forage on the naturally occurring net-fouling-organisms so that a baseline of growth and survival for comparison with fed groups could be obtained.

(2) Henderson Inlet - salmon/prawn polyculture - surface pen reared. Zero-aged chinook salmon were reared together with prawns in the same net-pen. These prawns had excess fish food pellets and fish excretion as possible food sources. Fish were fed Oregon moist pellets at a rate of 3 percent of body weight per day.

(3) Henderson Inlet - commercial fish pellets - surface pen reared. These prawns received a ration of 3/16 inch Oregon moist pellets via a plastic tube onto a feeding tray laying horizontally upon the bottom of the net pens.

(4) Henderson Inlet - non supplemented - benthic cage reared.

An experimental treatment group was positioned by divers under high density (0.9 fish per ft^3 or 2.0 lb/ft^3 estimated at harvest) coho salmon pens where excess feed and fish feces could be utilized as a food source. The control treatment group was located on a similar type of substrate in an area outside the direct effects of the salmon pens. These were four replicate for each of the two treatment groups.

The surface-pen groups of post-larvae and yearlings were fed daily ad libidum. Periodic (usually daily) checks for mortalities, exuvia and excess food were performed.

Experimental Monitoring Methods

The specific parameters, depth and frequency of measurement are presented in Table 2. At Henderson Inlet water temperatures and salinity were determined by using a Kahlsico model R55-3 temperature and salinity probe. Dissolved oxygen was recorded from the same depths by the use of a YSI model 51A. dissolved oxygen meter. Sampling at Henderson Inlet took place ±30 minutes of the daylight low tide.

At Clam Bay water temperature was recorded with an Applied Research Austin SD-20 Scanner probe at midday. A secchi disc was used at both sites to measure light penetration (visibility).

As part of an environmental impact study (Pease, 1975) at Henderson Inlet several water quality and benthic sediment parameters were monitored on a monthly and bi-monthly basis, respectively. Of the various parameters examined the following appeared to have direct bearing on prawn growth and survival.

Chlorophyll *a*

Values were obtained using a Turner fluormeter Model III and the methods given by Strickland and Parsons (1972) with modifications according to Pease (1975).

Sediment Carbon and Nitrogen

Core samples (2.8 cm ID, 3.0 cm long) were collected by divers in a random pattern near the benthic cages. Samples were immediately

Table 2. Depth and frequency of environmental measurements

Parameter	Frequency at Henderson Inlet	Depth	Frequency at Clam Bay	Depth
water temperature	daily	0.5 m & 1.0 m above the bottom	daily	0.5 m
salinity	monthly	0.5 m & 1.0 m above the bottom	-	-
visibility	daily	N. A.	daily	N. A.
dissolved oxygen	daily	2.0 m & 1.0 m above the bottom	-	-
chlorophyll a	monthly	1.0 m	-	-
sediment carbon	bi-monthly	N. A.	-	-
sediment nitrogen	bi-monthly	N. A.	-	-

N. A. = not applicable

frozen for later analysis at the Weyerhaeuser Research Laboratory at Harbor Island, Seattle. Initially, carbon samples were analyzed with an AMICO C-H analyzer, nitrogen samples by a Kjeldahl digestion technique. Samples collected after June 1974 were analyzed using a Carlo Erba (C-N-H-O) analyzer.

Growth Sampling

All surviving prawns and a subsample of 50 fish from each polyculture pen were measured at each sampling interval.

Juvenile Prawns

At the initiation of the experiment carapace lengths were recorded to the nearest 0.1 mm by using a binocular dissecting microscope equipped with a disc micrometer. Measurements were made to the mearest 0.1 mm. By October the animals were large enough to be measured with calipers (to the nearest 0.5 mm). Individual weights were not recorded at the 1st or the 1st through 3rd measurement periods at Clam Bay and Henderson Inlet respectively. Instead mean weight for these periods was estimated using a formula given by Butler (1964), i.e., Log W = 2.93148 Log L - 3.07787. Thereafter individual wet weights were recorded on an electronic top loading balance to the nearest 0.01 gram. Measurements were made after two months for the first period and monthly for the remainder of the experiment.

Yearling Prawns

Carapace lengths were recorded with calipers to the nearest 0.5 mm. Individual wet weights were taken on an electronic top loading balance. Bi-monthly sampling intervals were selected since wild populations of

this age increased at the rate of only 1 mm/month (Butler, 1964). More frequent measurement would not yield any useful information.

Chinook Salmon

The zero-aged chinook salmon were sampled on a monthly basis. Fork lengths and total sample group weights for fish in each of the three replicate pens were recorded.

Analysis and Presentation of Experimental Results

Raw data were analyzed by the use of Biomed program series on the University of Washington's CDC 6400 computer system. Prawn growth data were analyzed using one way analysis of variance for initially similar sized groups while analysis of covariance programs were utilized for initially dissimilar sized groups. Regressions were derived from the analysis of covariance programs.

Chi square tests were used to assess mortality between treatment groups (after fulfilling the requirements of a heterogeneity chi square for the within groups variation).

Interval (average) growth rate was calculated for length and weight by the following formula:

$$P_I = \frac{X_o - X_t}{n}$$

Specific growth rate (grams of weight change per gram of body weight per day) was calculated by the formula:

$$P_S = \left(\frac{X_t}{X_o}\right)^{1/n} - 1 \quad \text{or} \quad P_S = \frac{(\ln X_t - \ln X_o)}{n}$$

X_o = mean initial size

X_t = mean final size (on nth day)

n = number of elapsed days in a given interval

p = percent gain expressed as a decimal

Results of sediment analysis were expressed as percent by weight.

RESULTS

Hydrography

Data presented in this section covers the period of May to December 1974 at Henderson Inlet; and from May 1974 to March 1975 at Clam Bay.

Temperature

In early June mean weekly water temperatures at both sites began to rise from the 9 $\pm$ 1° C observed throughout May (Figs. 3, 4, 5). At Henderson Inlet mean 0.5 m water temperature rose about 5° C in one week. There was a synchronous but more gradual increase at Clam Bay, i.e., 4° C increase over a three week period.

At both sites mean weekly water temperatures remained fairly constant for the summer months and began to decline by September. Although Henderson Inlet means (surface and bottom) and Clam Bay means (surface) generally followed the same trend Henderson Inlet surface waters averaged one to three degrees warmer until October (Fig. 2).

The overall 0.5 m mean water temperatures for each site are given in Table 3; the entire period is further partitioned into summer versus fall. Henderson Inlet was warmer overall, particularly in the summer months when mean temperatures at 0.5 m water depths averaged 2.5° C warmer than Clam Bay.

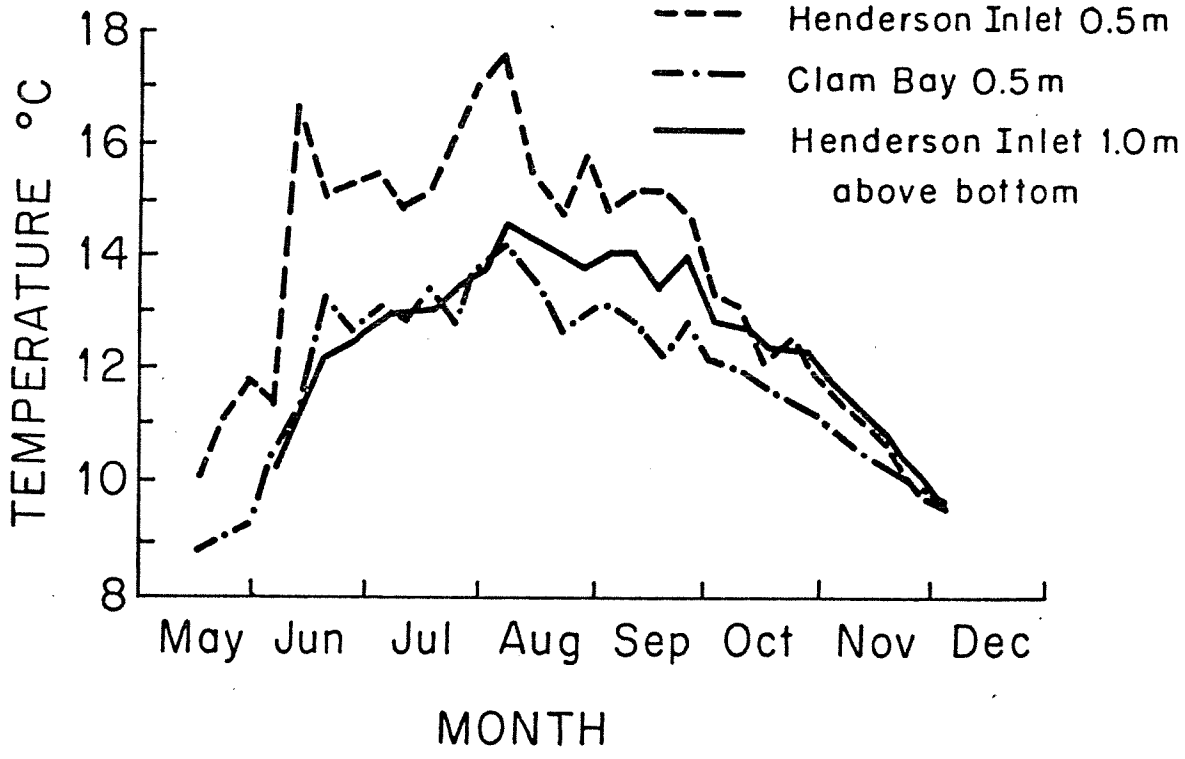

Fig. 2. Weekly mean water temperatures for Henderson Inlet 0.5 m below surface, 1.0 m above bottom, and at Clam Bay, 0.5 m below surface from May to December 1974.

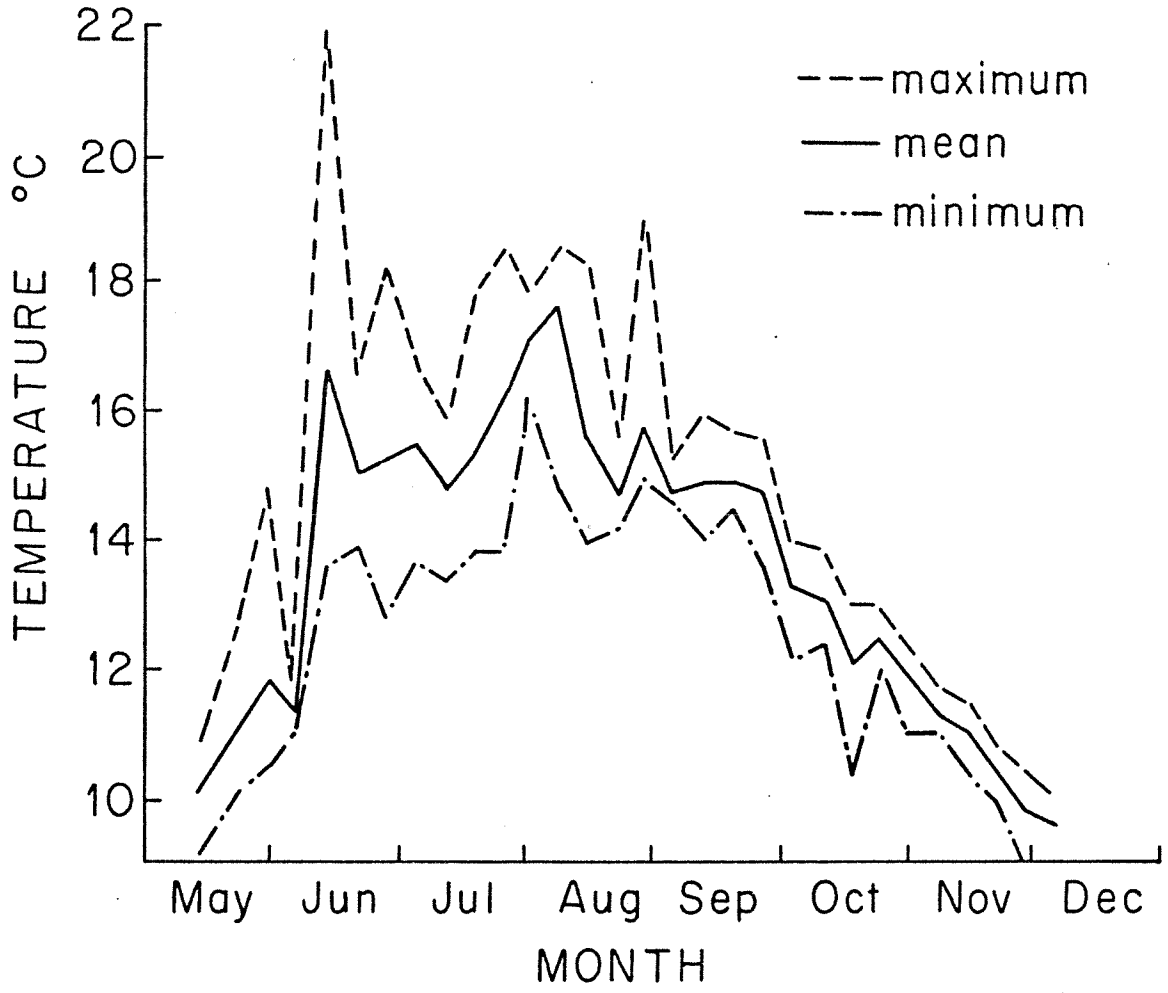

Fig. 3. Weekly maximum, mean and minimum 0.5 m water temperature at Henderson Inlet, May to December 1974.

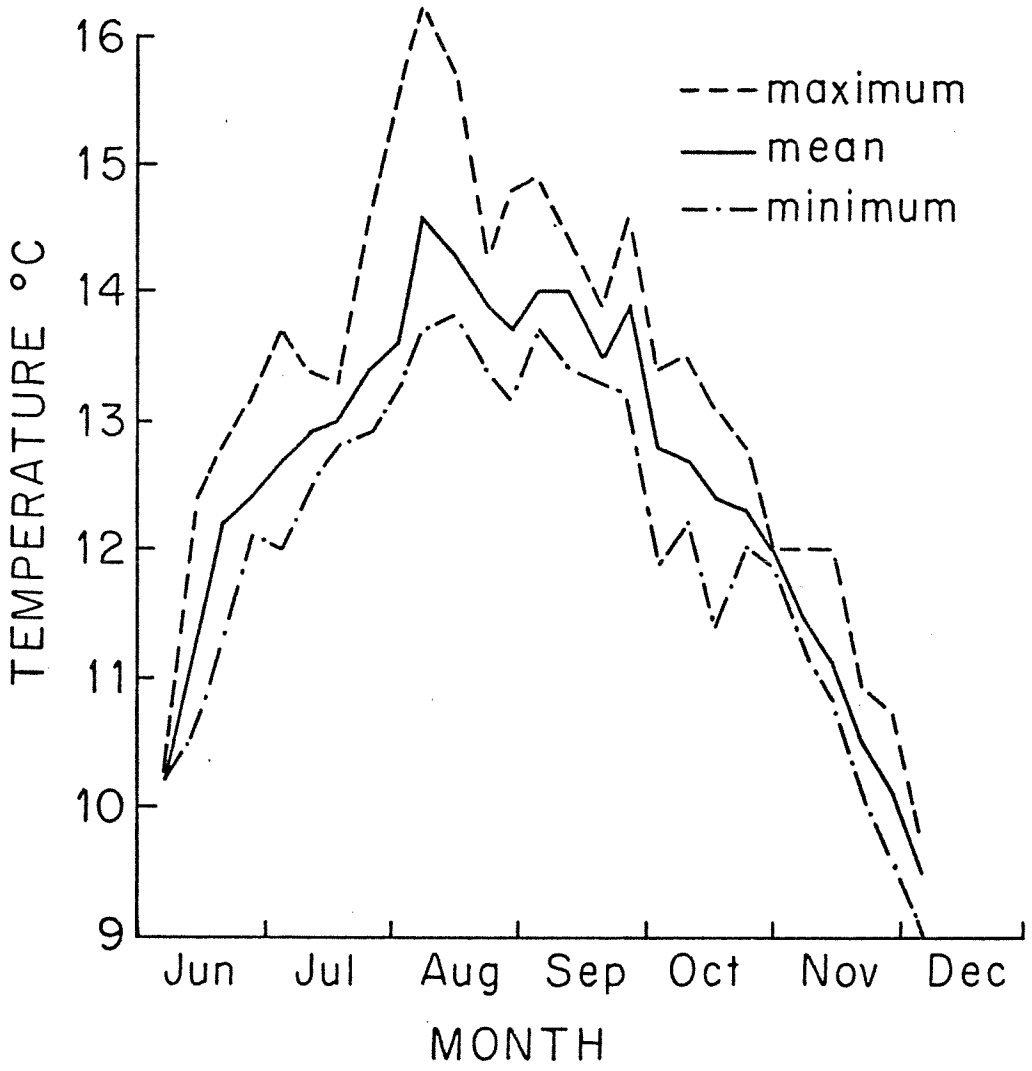

Fig. 4. Weekly maximum, mean and minimum 1.0 m above bottom water temperatures at Henderson Inlet, June to December 1974.

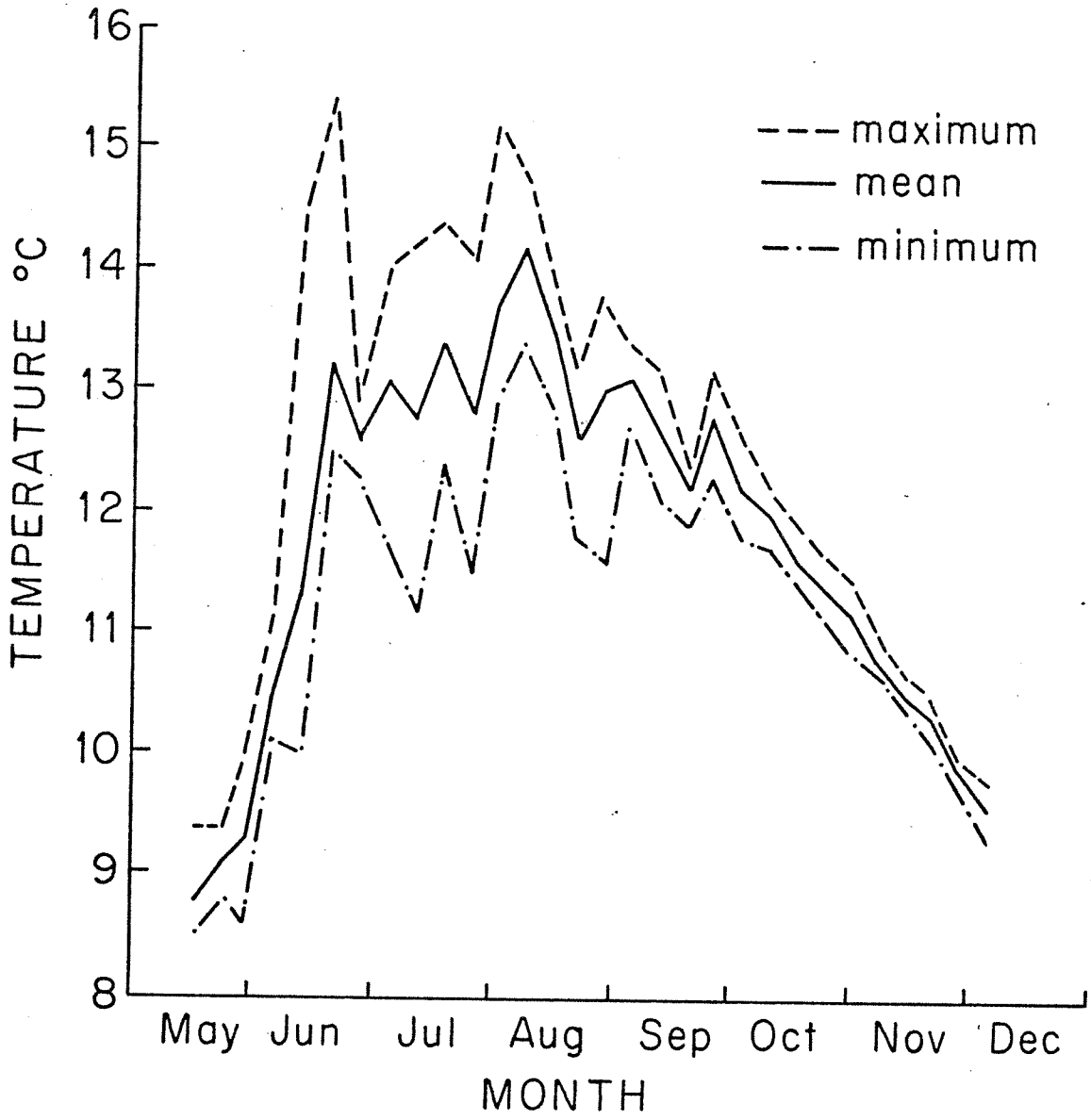

Fig. 5. Weekly maximum, mean and minimum 0.5 m water temperature at Clam Bay, May to December 1974.

Table 3. Mean .5 m water temperatures for the specified time period and site.

	Henderson Inlet	Clam Bay	Net difference at Henderson Inlet
5/31 - 12/5 overall mean	13.9	12.1	1.8+
range	9.0-21.9	9.3-15.2	
5/31 - 9/26 summer only	15.3	12.8	2.5+
range	11.0-21.9	10.0-15.2	
9/27 - 12/5 fall only	11.5	10.9	0.6+
range	9.0-12.4	9.3-12.6	

Short term fluctuations caused by solar radiation during summer months was observed in Henderson Inlet surface waters (Fig. 3) while bottom waters at the same site (Fig. 4) and Clam Bay surface waters (Fig. 5) were not as variable.

Salinity

Although salinity was not monitored at the Clam Bay site, previous work (Moring, 1973) has shown very similar salinity regimes for Clam Bay and Squaxin Island, a salmon farming site within four miles of Henderson Inlet. In that study salinity fluctuations never exceeded 1°/oo in 24 hours at Squaxin Island where salinity conditions were described as relatively stable. Squaxin Island salinities ranged from 27 to 31°/oo over a ten month period.

Similar results were obtained at Henderson Inlet in this study since 0.5 m salinity (Fig. 6) ranged from 28.4 to 31.7°/oo during the period of expected higher salinities (late summer and fall). Bottom salinities at Henderson Inlet were slightly higher (<1.0°/oo) than surface values and ranged from 29.3 to 31.7°/oo (Fig. 6).

Dissolved Oxygen

Dissolved oxygen was monitored regularly at the Henderson Inlet site only. Dissolved oxygen at 2 m and the bottom parallelled one another throughout the study (Fig. 7). Weekly maximum and minimum dissolved oxygen for 2 m and bottom waters are given in Figs. 8 and 9.

The overall trend was a gradual decline from saturation values experienced in May to a plateau near 7 ppm during August to October. A slight rise was seen in November at both surface and bottom. The lowest

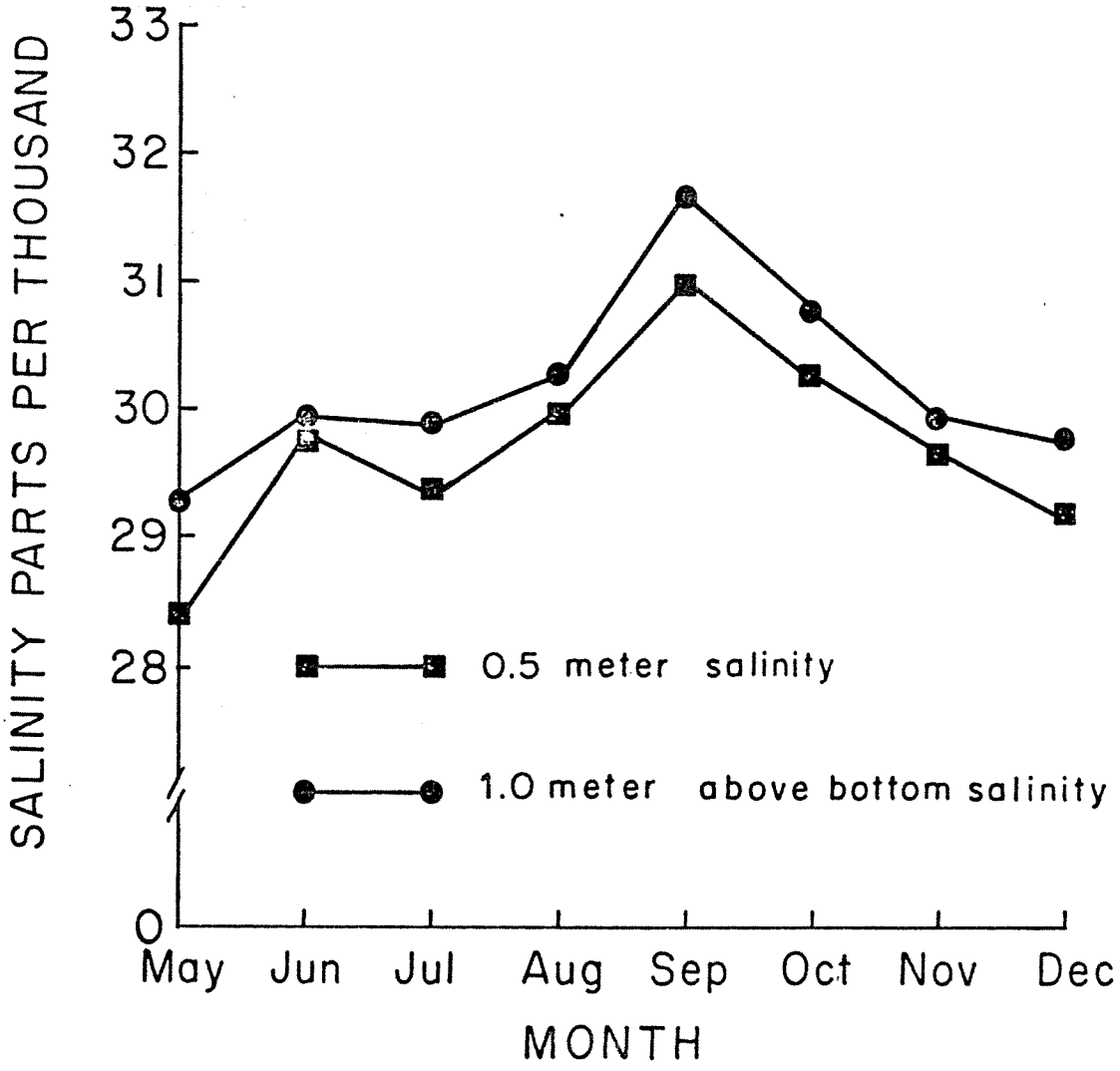

Fig. 6. Monthly 0.5 m and 1.0 m above bottom salinity at Henderson Inlet, May to December 1974.

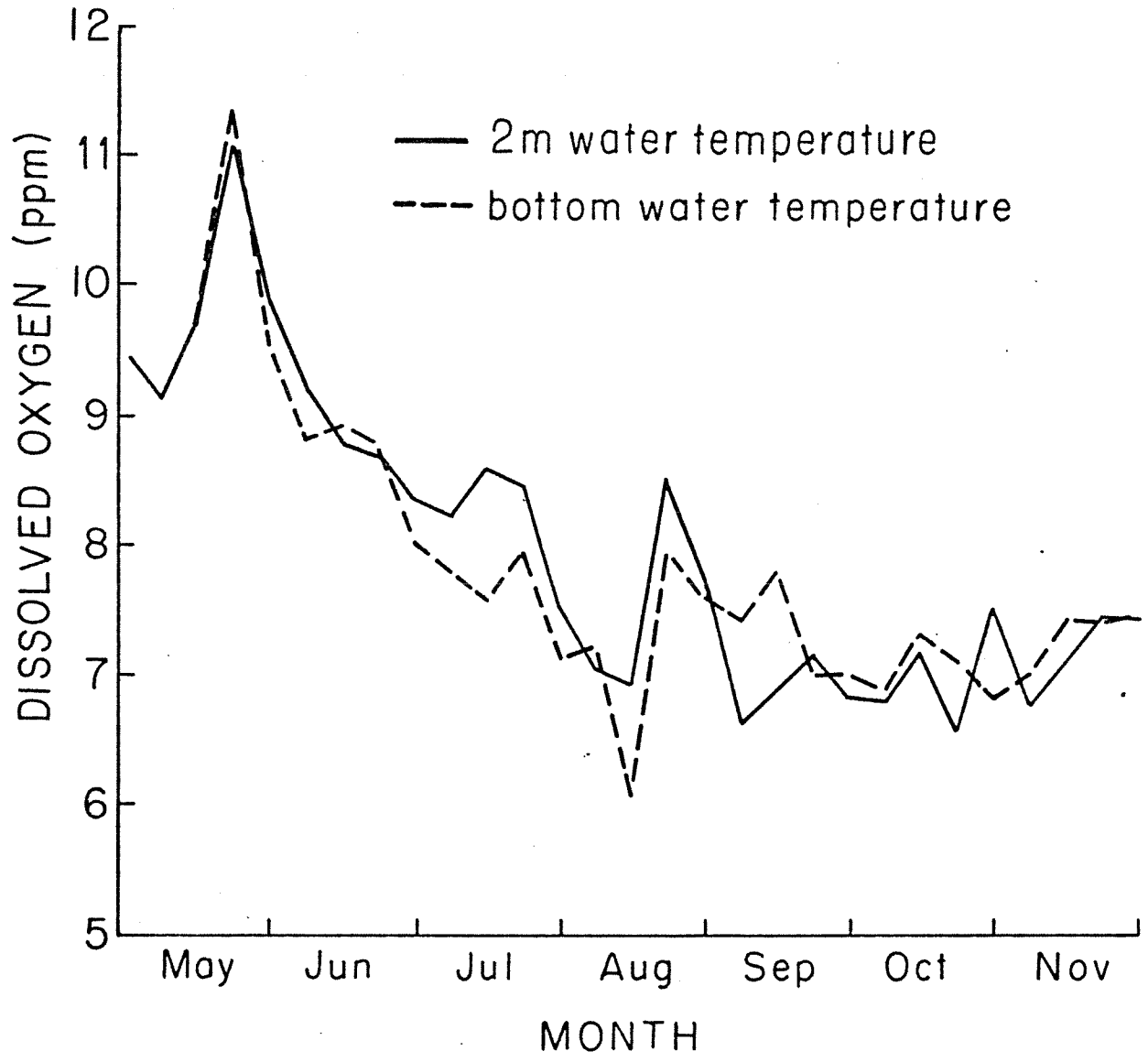

Fig. 7. Weekly mean 2.0 m and 1.0 m above bottom dissolved oxygen at Henderson Inlet, May to December 1974.

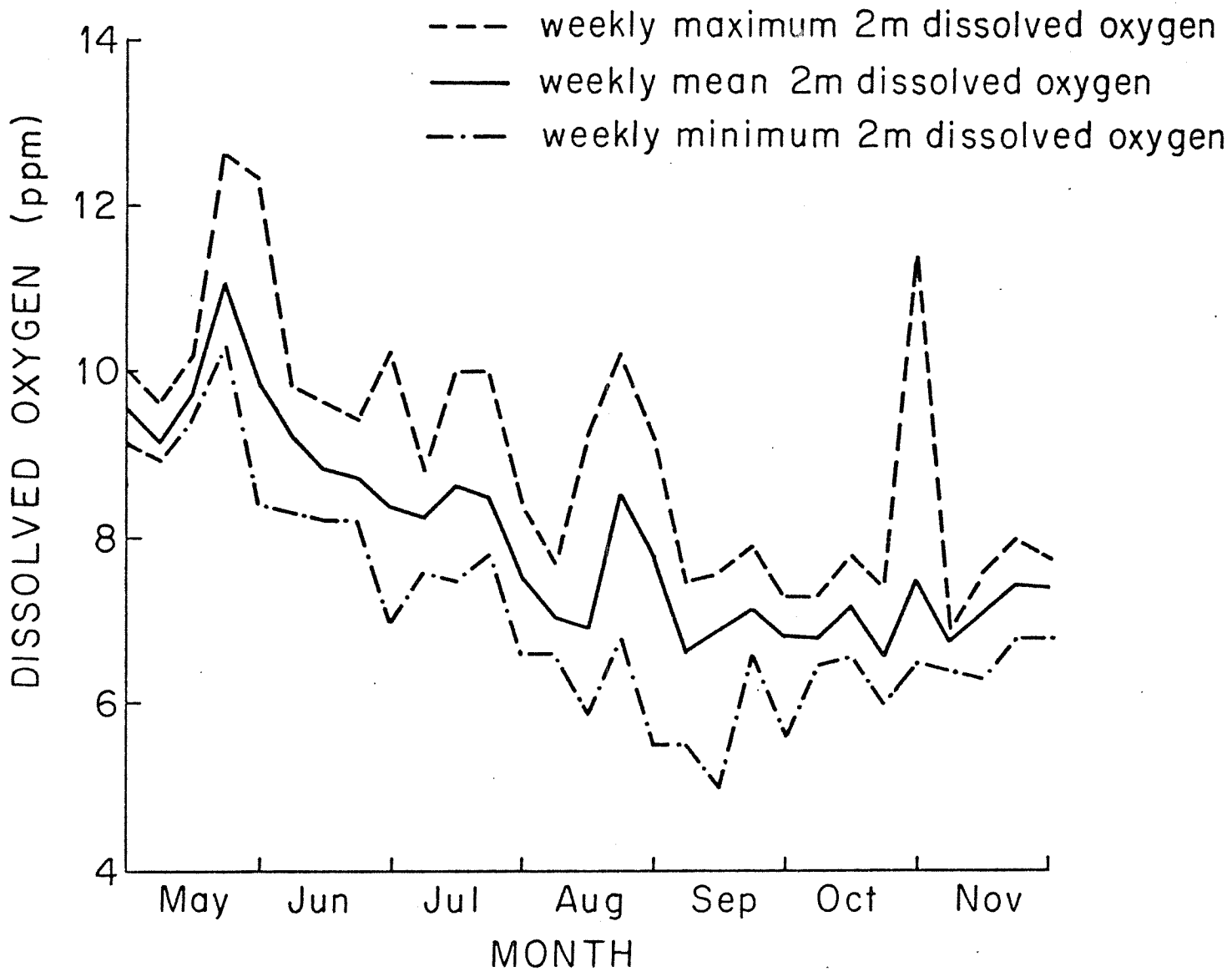

Fig. 8. Weekly maximum, mean, and minimum 2 m dissolved oxygen concentration at Henderson Inlet, May to December 1974.

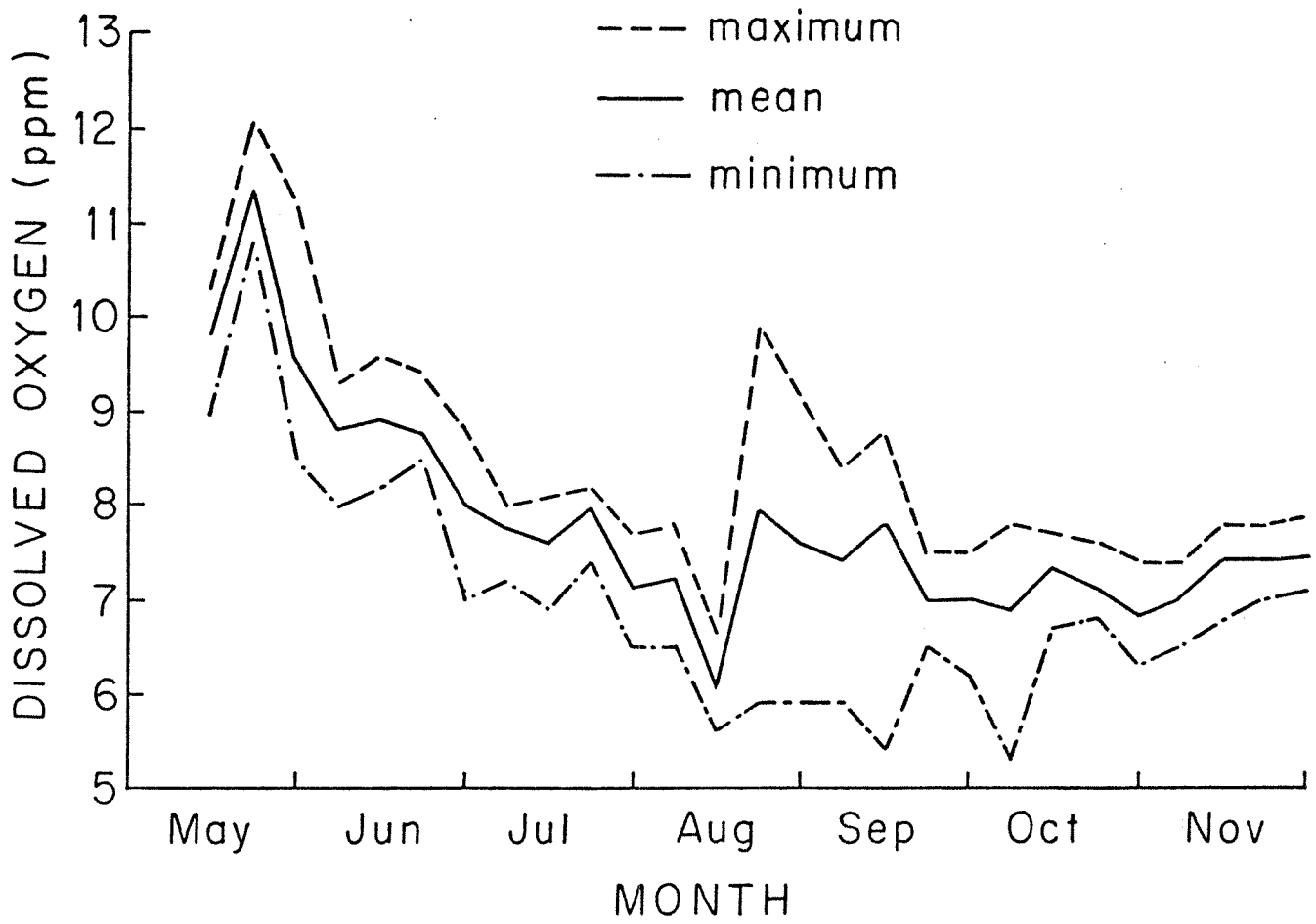

Fig. 9. Weekly maximum, mean, and minimum 1.0 m above bottom dissolved oxygen concentration at Henderson Inlet, May to December 1974.

dissolved oxygen level recorded at 2 m was 5.0 ppm in mid-September. This low value occurred at the end of an extensive plankton bloom that began several weeks before. Bottom dissolved oxygen levels reach minimums of 5.6, 5.4 and 5.3 ppm in mid-August, September and October, respectively.

Light

During the inter-site comparison (May to December, 1974) mean bi-weekly visibility ranged from 1 to 4 m less at Henderson Inlet than at Clam Bay (Fig. 10). Decreasing and increasing light penetration levels at both sites were caused in part by plankton blooms. Figure 10 shows that monthly values of chlorophyll *a* at Henderson Inlet increased from July to September, peaking at a period of lowest light penetration during a dense plankton bloom. During other periods chlorophyll *a* values were much lower and visibility was increasing (particularly in the late fall). Bi-weekly means exhibit a gradual increase at Henderson Inlet in the fall. At Clam Bay bi-weekly means increase from January to March, 1975. Ranges of bi-weekly means show similar trends at both sites. Large fluctuations, particularly at Clam Bay, occur in summer months. This is followed by a period of narrow ranges in fall and early winter. At Clam Bay, where data was collected for three additional months, the final trend is toward increased variation in ranges.

Sediment Carbon and Nitrogen

Sediments beneath the salmon pens nearly doubled in the amounts of total nitrogen and total carbon during this experiment. Values obtained from the control area fluctuated but remained near the initial levels

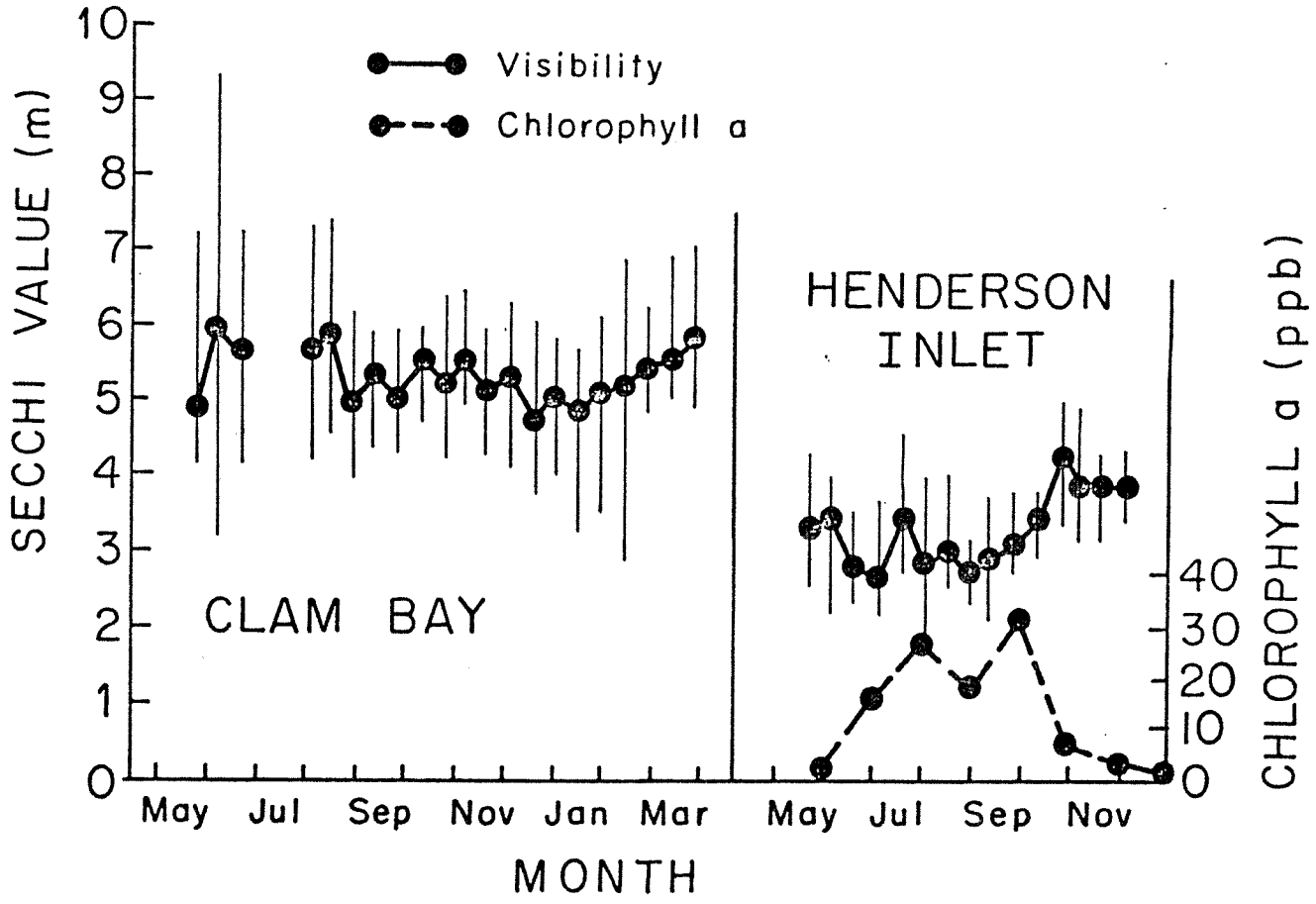

Fig. 10. Mean and range of biweekly visibility at Clam Bay and Henderson Inlet with 2 m chlorophyll *a* concentration at Henderson Inlet.

(Figs. 11 and 12). Maximum amounts of both parameters were reached by early December 1974 and declined slightly by March 1975 (at experiment termination).

Survival

Juveniles

Soon after initiation in early July a low level of mortality was observed in juvenile prawns at both sites (Fig. 13). At Henderson Inlet dead or moribund individuals had singular, necrotic lesions, dark in the center and often surrounded by inflamed, reddish tissue.

Only five percent mortality occurred at Clam Bay versus 18 percent at Henderson Inlet during the month of July. However, the slow rate of mortality at Clam Bay continued through the summer and fall (November 27, 79.3% survival).

From August 29 through September 12, surface water at Henderson Inlet was affected by the presence of plankton bloom consisting predominately of the dinoflagellates Ceratium sp. and Peridinium sp. During this period 45 percent of the initial number of prawns died as did many of the salmon in the adjacent net pen farm. Dissolved oxygen levels were monitored day and night but oxygen depletion did not occur. Inspection of the gills revealed foreign matter on the lamellae. Microscopic examination showed no readily identifiable fragments in this material except an occasional non-motile rod shaped bacteria. The plankton did not appear to be clogging the gills, rather it appeared to be promoting the production of a mucus like material possibly due to the abrasive nature of the dinoflagellate's spine-like projections. After

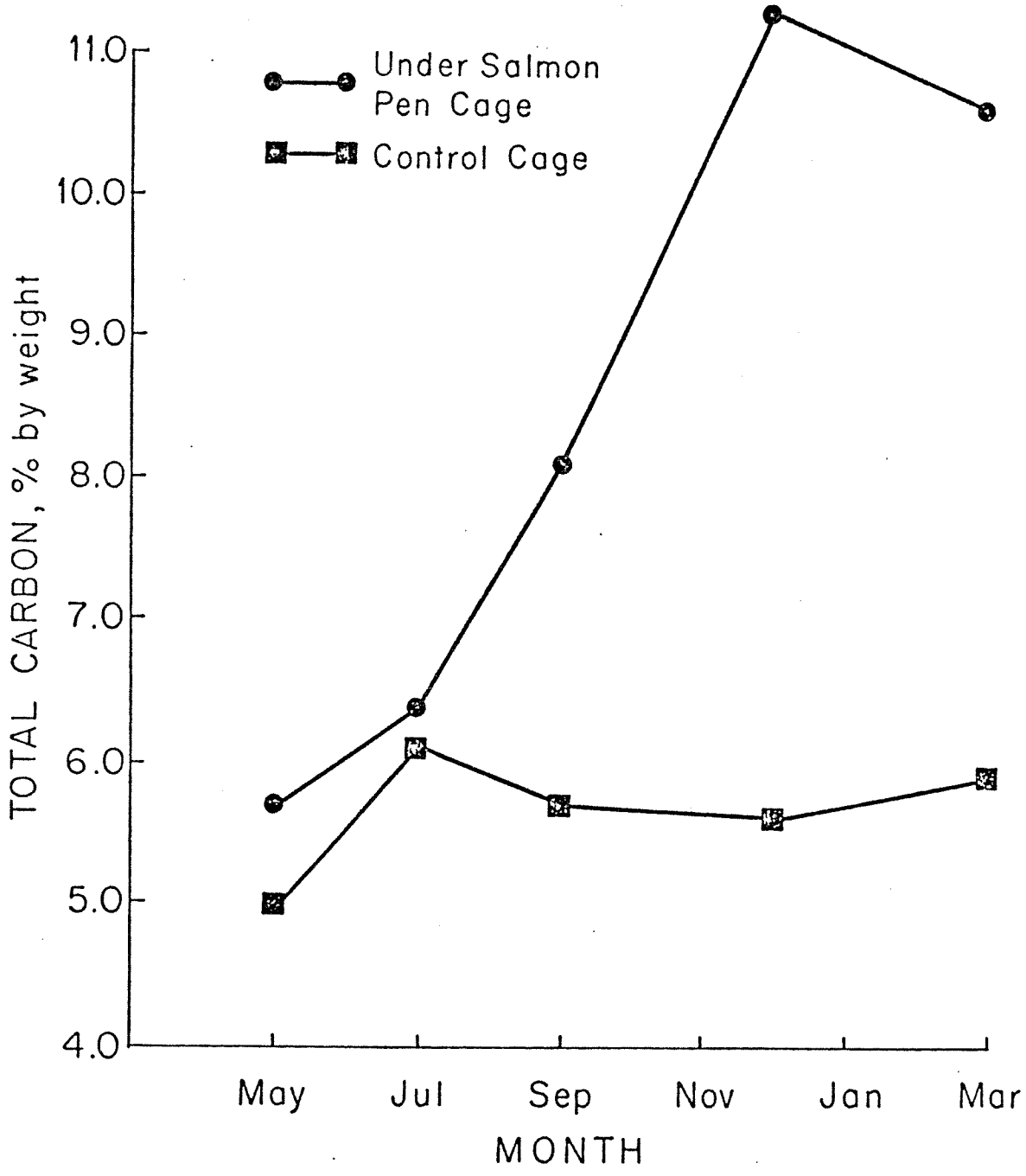

Fig. 11. Total carbon in sediments cored from beneath salmon pens and control areas at Henderson Inlet, May 1974 to March 1975 (from Pease, 1975).

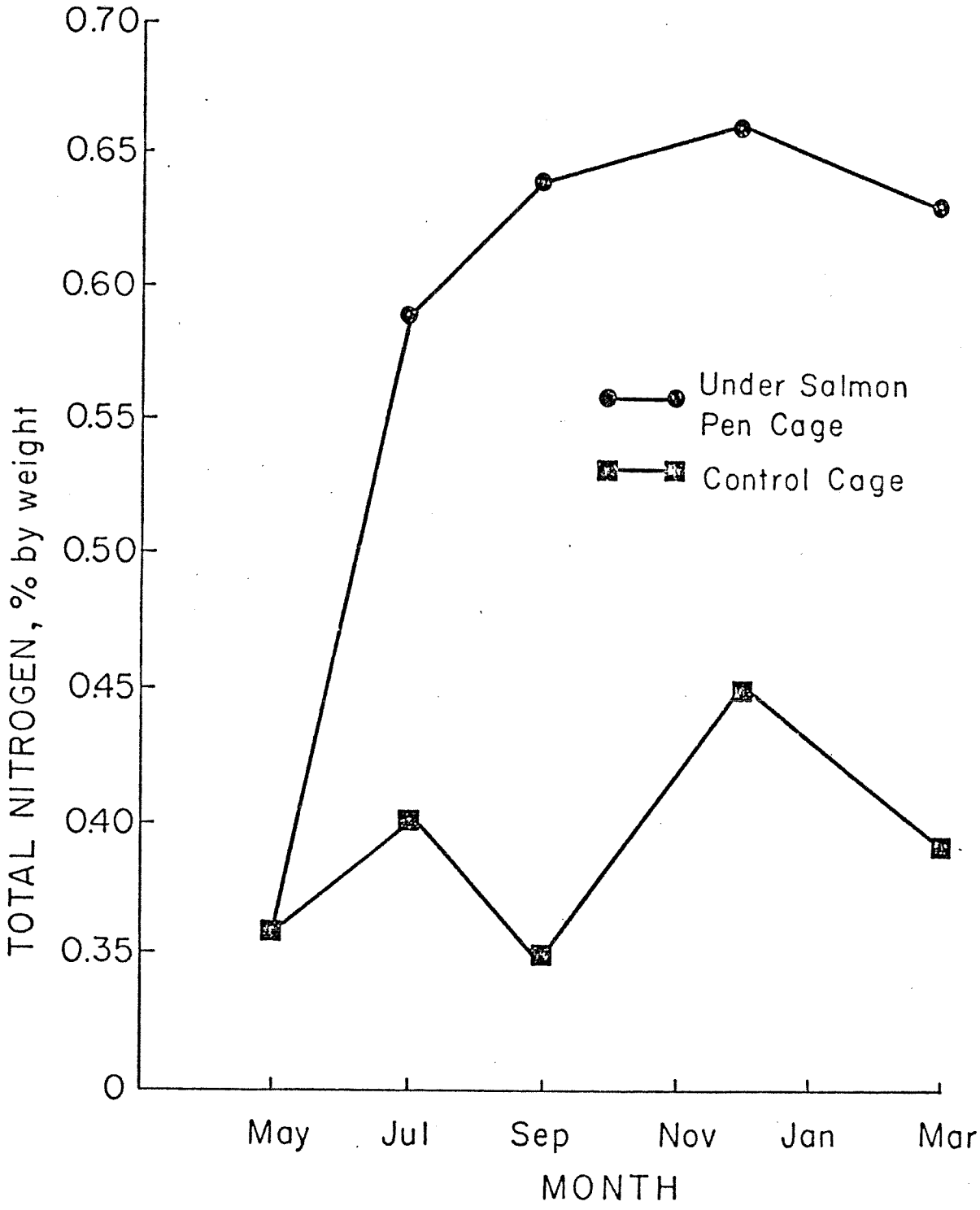

Fig. 12. Total nitrogen in sediments cored from beneath salmon pens and in control areas at Henderson Inlet from May 1974 to March 1975 (from Pease, 1975).

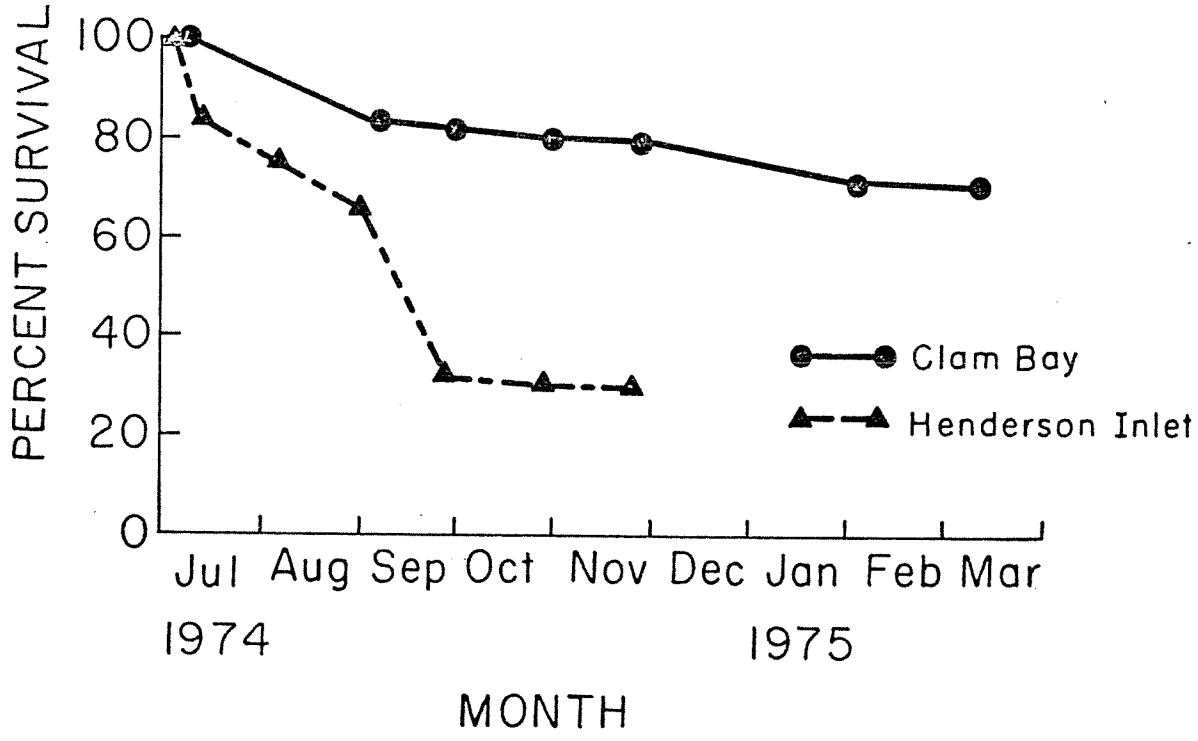

Fig. 13. Survival of juvenile prawns from July 1974 to March 1975.

this period survival remained relatively constant until termination in late November (30.3% survival).

Juvenile prawns at Henderson Inlet did not seem to be affected by the fouling problems that plagued the surface reared yearlings. Higher molt frequency did not allow epicommensals to become established upon their exoskeleton or gills.

A chi square analysis of survival as of November 27, 1975, demonstrates significantly higher survival ($X^2 = 67.2$, $0.025 < P < 0.05$) for juveniles reared at Clam Bay versus Henderson Inlet.

Yearlings - Clam Bay

Unsupplemented and clam processing waste fed groups displayed similar survival during the first measurement interval with a 3.9 percent loss in each (Fig. 14). During the second period both groups were subject to a slight increase in the number of mortalities. A significant difference ($X^2 = 4.16$, $0.025 < P < 0.05$) in survival existed between the two groups by the end of this interval in September; the clam processing waste fed group had 83.6 percent survival, while the unsupplemented group was at 77.4 percent survival. Thereafter relatively few mortalities were encountered and both groups declined slightly in numbers until termination in late March. Final survival was 78.6 percent for the clam fed group versus 66.7 percent for the unsupplemented group, which had apparently maintained itself on net fouling and pelagic organisms although growth was very slow compared to other treatment groups (see growth section).

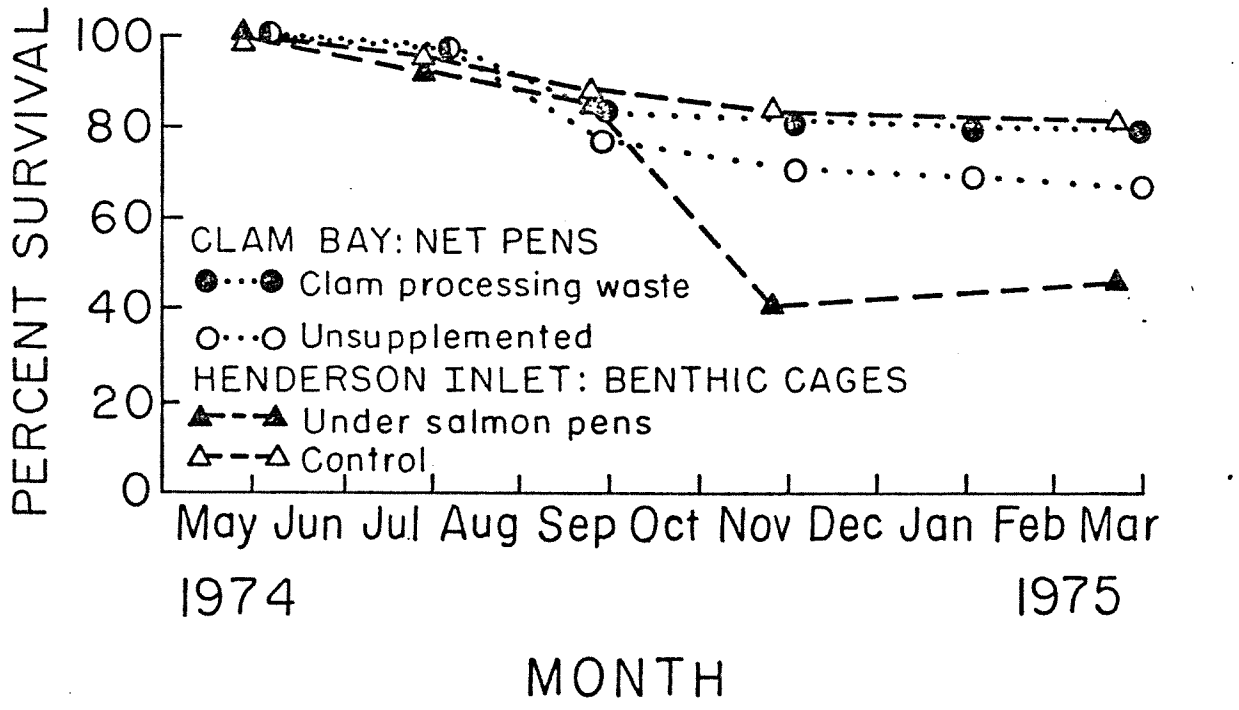

Fig. 14. Survival of yearling prawns from May 1974 to March 1975.

Yearlings - Henderson Inlet Surface Pens

Experiments at Henderson Inlet surface waters were initiated on June 14, 1975. Within two days several prawn mortalities were observed and thereafter the number of dead increased daily. Remaining prawns did not consume their ration, seemed lethargic and failed to molt. Fouling organisms, particularly the hydroid Obelia sp. and the suctorian protozoan Ephelota gemmipara became established upon the entire prawn exoskeleton with heaviest concentrations on the periopods.

On July 15 all surface reared prawns were treated with an eight hour dip in 25 ppm formaldehyde and 0.1 ppm malachite green solution. Aeration was provided while dissolved oxygen and temperature were monitored. A twenty percent mortality occurred during treatment but virtually all fouling organisms were eliminated. Upon re-introduction to the net pens most prawns fed actively and displayed normal self-cleaning behavior. Since the source of the fouling organism (net-pens and floats) were still holding large numbers of protozoan covered hydroids fouling began almost immediately followed by increasing mortality. During this post-treatment period several prawns were observed with black gills (melanization of the gills) which is not a disease in itself but a generalized sing of internal disorders, diseases or degraded environmental conditions (Sindermann, 1974; Lightner et al., 1975). In a further attempt to sort out the cause(s) of the mortality all survivors (n=49) of one of the fish food fed replicate groups were sunk to the bottom in a benthic cage on July 2. They became active and although many still had fouling organisms the rate of mortality was greatly reduced. By September only four mortalities had occurred (92% survival) while those groups left in surface net pens suffered complete mortality.

Benthic Cage Yearlings

At Henderson Inlet survival was 86 and 88 percent for under salmon pen and control groups after 120 elapsed days (Fig. 14).

During the third measurement interval (October, November 1974) survival in the under salmon pen group was reduced to 40.5 percent which was significantly less ($X^2 = 24.0$, $P < 0.001$) than the control groups (83.3% survival). Only two of the four under salmon-pen cages were sampled at this time due to the extremely poor visibility (< one foot) that made locating these cages by diving impossible. Surrounding areas, including the control-cage areas, were not affected by this turbidity. Survival levels remained relatively constant after this interval until termination in March 1975. All four under salmon-pen-cages were sampled at this time which explains the slight rise of percent survival seen in Fig. 14. There was no difference ($X^2 = 2.06$, $0.50 < P < 0.75$) in survival within replicates of the under salmon-pen group which suggests the previous measurement, based on two pens, was representative of the survival of all four pens. Survival was significantly higher in the control-cages ($X^2 = 33.14$, $P < 0.001$) than for the under salmon pen cages at project termination. The control group survival figure of 81.0 percent represents the highest survival rate of any yearling group at both sites, slightly surpassing the Clam Bay clam processing waste fed group (78.6% survival).

During the third measurement interval, when under salmon-pen groups had the sudden drop in survival, sediment cores from the nearby transect had the maximum amount of total carbon and nitrogen observed during this study, 11.26 and 0.66 percent respectively (Figs. 11 and 12). In contrast

the levels of these parameters for the control-cages did not rise appreciably. The chemical changes were accompanied by hydrogen sulfide production and gross faunal changes which can be attributed to increased feeding operations in the overlying salmon farm. Large numbers of the polychaete worm Capitella capitata, generally recognized as an indicator of pollution when found dominating the benthic community (Reish, 1970) were found partially covering the sides and bottoms of the benthic-cages beneath the salmon-pens in November. Highest production of ammonia from the salmon wastes (Pease, personal communications) and the lowest values of dissolved oxygen observed (5.3 to 6.6 ppm for all sampled areas) were further indicators of poor water quality during the October-November period.

Growth

Juveniles

Net-pen reared juvenile prawns were homogeneous in terms of initial carapace length both within replicates at each site and between sites ($0.25 < P$ and $0.10 < P < 0.25$ respectively).

By late November, when the Henderson Inlet juvenile experiments were terminated, the Clam Bay groups were significantly larger in carapace length and weight ($0.0005 < P < 0.001$). Growth rates shown in Tables 4, 5, and Figs. 15, 16 reveal highest rates of growth for Clam Bay juveniles from July to late September and for the entire culture period. Juvenile prawns reared at Henderson Inlet maintained relatively higher rates of growth than Clam Bay prawns in the fall and early winter. Consumption of mussels dropped markedly at both sites in the

Table 4. Average Carapace lengths and weights, ranges, standard deviation, interval and specific growth rates and number of surviving juvenile prawns at Henderson Inlet from July to November, 1974

sampling date	number surviving N	average carapace length * (mm)	standard deviation	carapace length range (mm)	average weight (gms)	standard deviation	weight range	interval weight increase per day	specific growth rate (g/g/day)
3 July 1974	135	5.5	1.74	2.0-11.0	0.12*	-	-	-	-
1 September	89	12.3	1.75	9.0-16.3	1.31*	-	-	0.0198	0.0406
28 September	43	14.6	1.52	11.4-18.1	2.16*	-	-	0.0315	0.0187
27 October	41	16.4	1.19	14.0-19.0	3.34	0.71	2.0-5.3	0.0407	0.0151
24 November	41	18.1	1.11	15.5-20.5	4.53	0.78	2.8-6.7	0.0425	0.0109

Average weight gain per day over the entire 144 days = 0.031 grams/day

*Calculated from the length-weight formula of Butler (1964).

Table 5. Average carapace lengths and weights, ranges, standard deviation, interval and specific growth rates and number of surviving juvenile prawns at Clam Bay from July 1974 to March 1975

sampling date	N	number of elapsed days	average carapace length (mm)	standard deviation	length range	average weight (gms)	standard deviation	weight range	interval weight increase per day	specific growth rate (g/g/day)
8 July 1974	135	0	5.2	1.66	3.0-11.1	0.10*	-	-	-	-
6 September	117	60	13.2	1.86	8.5-17.3	1.96	0.72	0.50-3.81	0.031	0.0508
30 September	111	84	16.3	1.72	12.8-20.1	3.08	0.84	1.48-5.17	0.046	0.0190
30 October	109	114	17.6	1.42	14.0-20.5	4.15	0.90	2.31-6.25	0.035	0.0099
27 November	108	142	18.9	1.21	17.0-21.5	5.07	0.90	3.12-7.31	0.032	0.0072
End of direct between site comparison - Post-larvae at this site were left in the same surface net-pens until termination in March 1975.										
2 February 1975	97	208	20.8	1.06	18.5-23.0	6.61	0.87	4.72-8.65	0.023	0.0040
10 March	96	244	21.2	1.14	18.5-23.5	7.13	0.95	5.17-9.00	0.014	0.0021

Average weight increase per day during the inter site comparison (142 days) = 0.035 grams

*Calculated from the length-weight formula of Butler (1964).

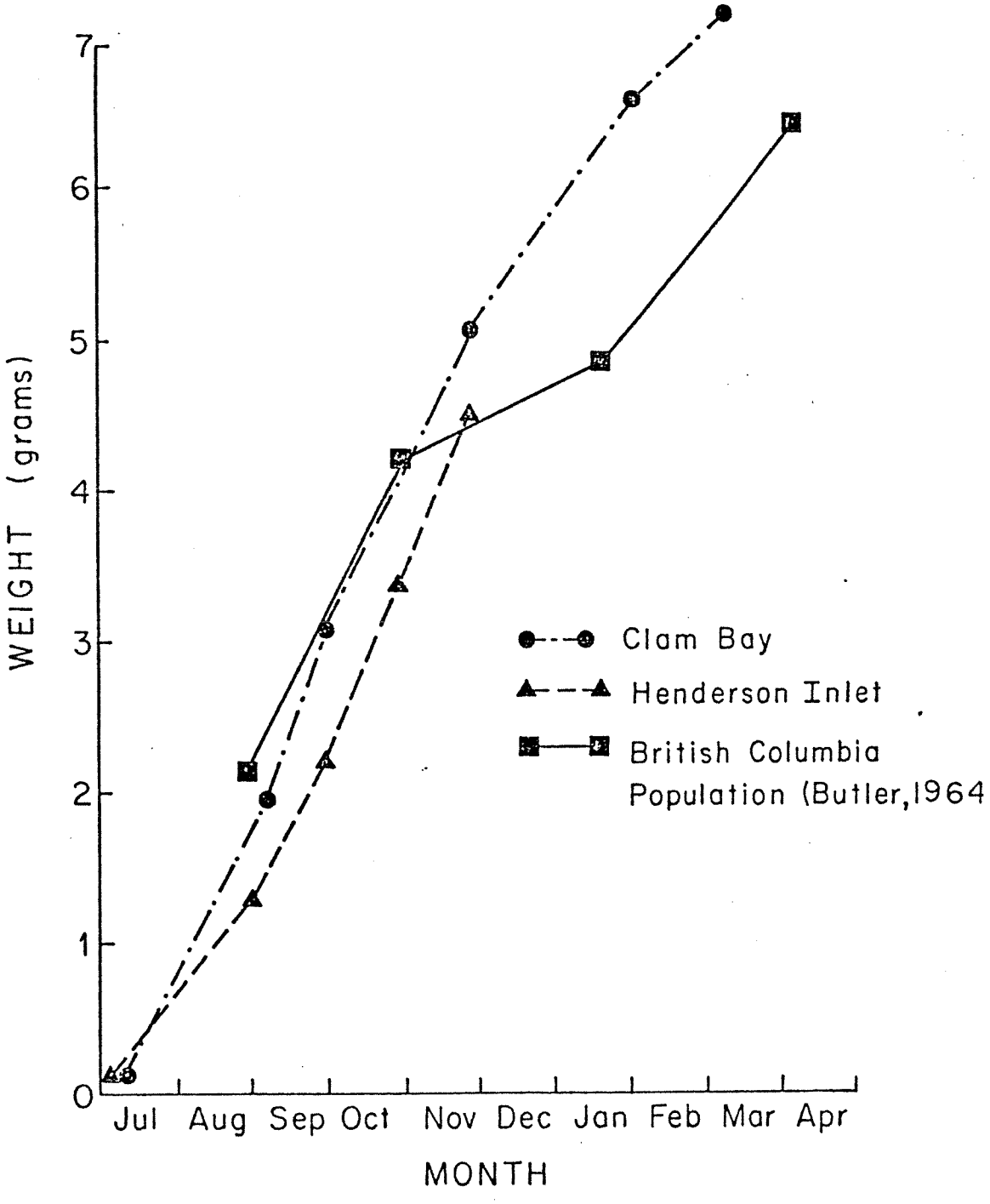

Fig. 15. Mean weights of juvenile prawns reared in net-pens at Clam Bay and Henderson Inlet, Washington compared to a British Columbia population (Butler, 1964).

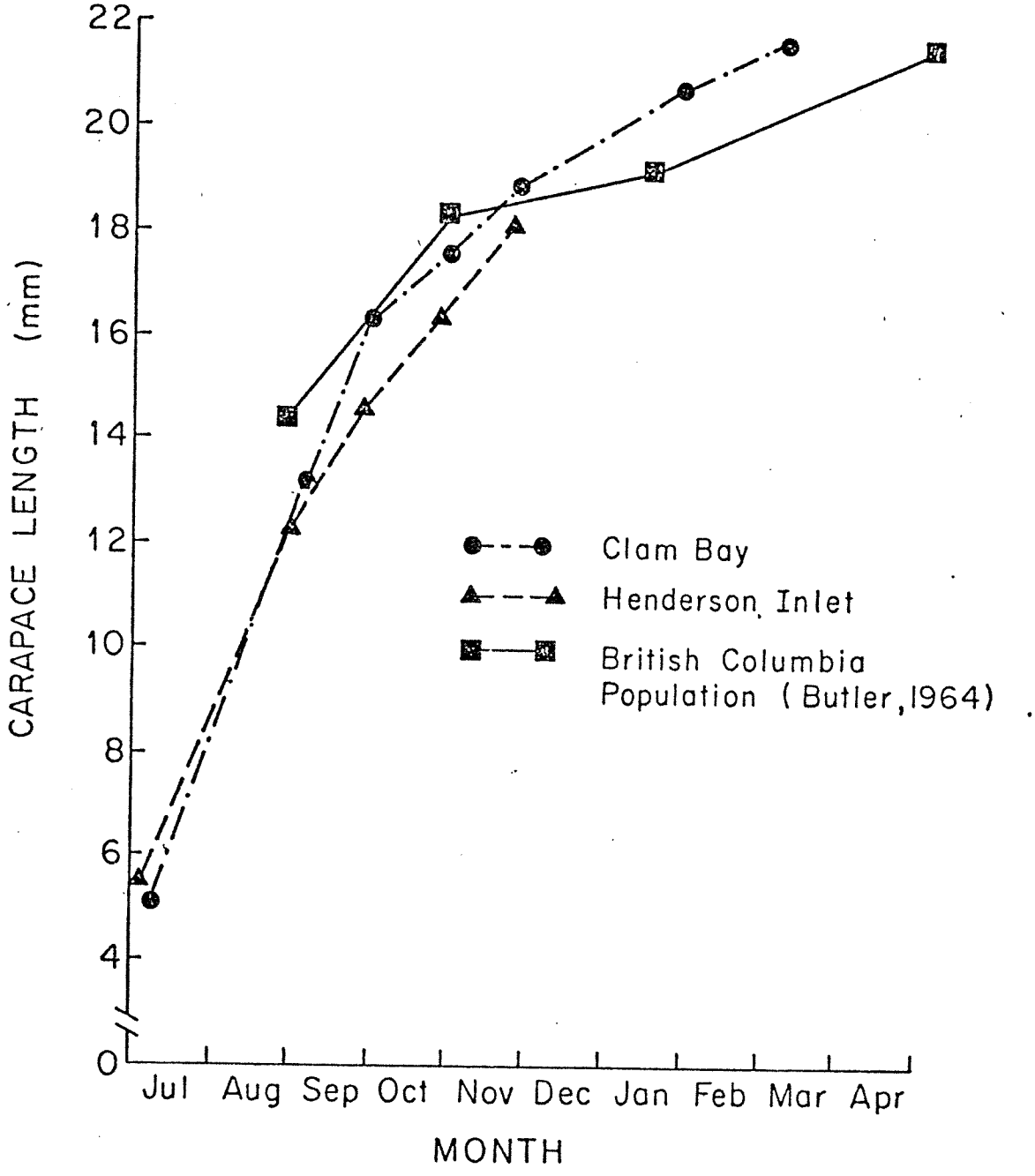

Fig. 16. Mean carapace length of juvenile prawns reared in net-pens at Clam Bay and Henderson Inlet, Washington, compared to a British Columbia population (Butler, 1964).

third week of October. A corresponding rapid drop in growth rate was not observed. Rather, prawns at both sites showed moderate decreases in growth rates until the experiment's termination in late November 1974 at Henderson Inlet and late March 1975 at Clam Bay.

Juvenile Growth Compared with that of a Natural Population

Analysis of covariance (Appendices 4 and 5) was used to show that length versus time and weight relationships are similar for Clam Bay juveniles and the reported British Columbia population (Butler, 1964). There was a significantly greater increase in terms of weight ($0.025 < P < 0.05$) for the Clam Bay juveniles that averaged 15 percent heavier than the natural population at the age of one year (Appendix 6). The natural population shows a decline in rate of weight gain from November to the end of January while the Clam Bay juveniles continue to grow at only a slightly decreased rate (Fig. 16).

Yearlings - Clam Bay - Net Pen Reared

Unsupplemented and clam processing waste fed prawns grew at essentially the same rate ($0.025 < P < 0.05$) during the first measurement period (Figs. 17, 18 and Table 6). At each measurement interval thereafter the clam fed prawns were significantly heavier and longer.

The unsupplemented group gained little weight after the first interval (Fig. 18) but did increase slightly in length (Fig. 17) throughout the experiment.

Stomach analysis of this unfed group at the experiment's termination revealed 60 percent had substantial amounts of brown unidentifiable materials. Also present in varying quantities were various algaes

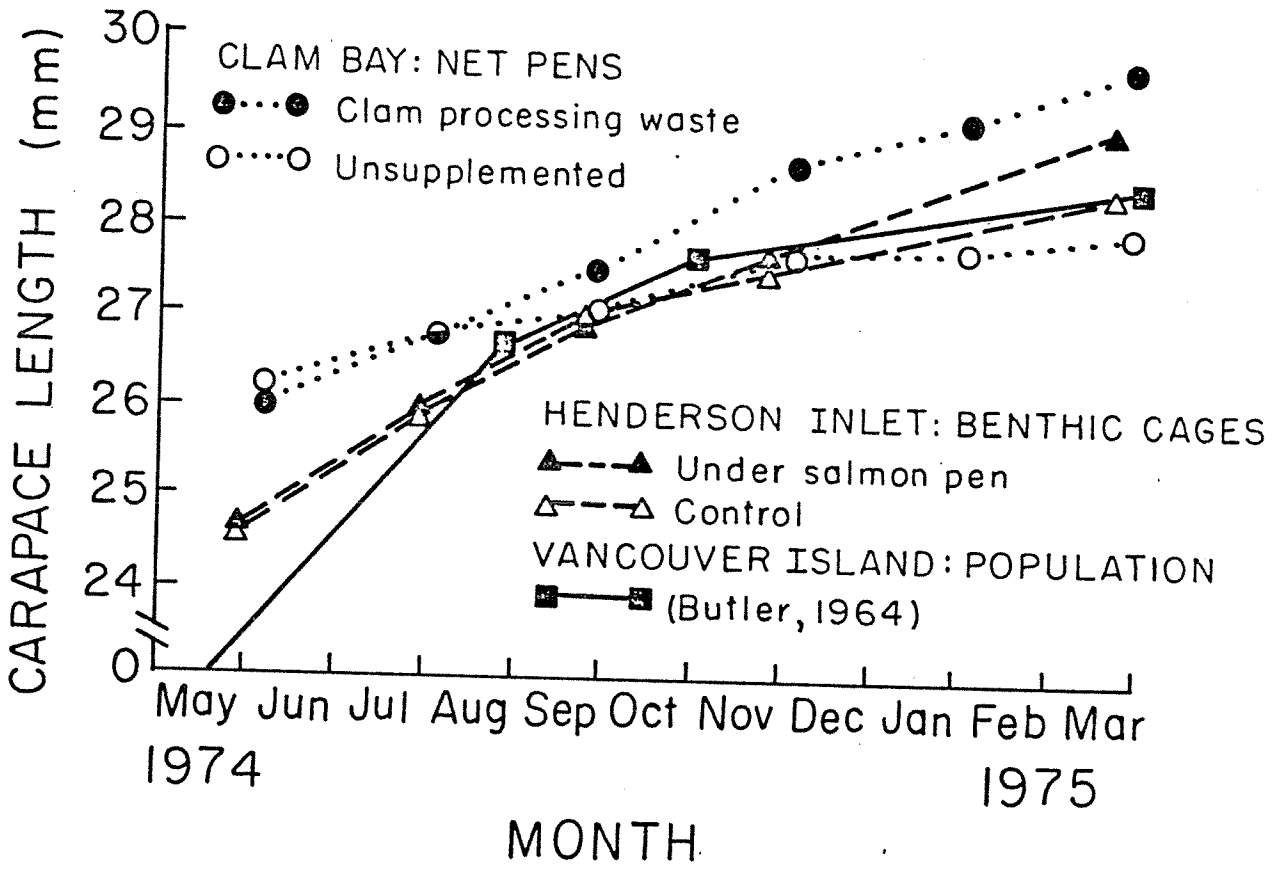

Fig. 17. Mean carapace lengths of yearling prawns reared at Clam Bay and Henderson Inlet, Washington, compared to a British Columbia population (Butler, 1964).

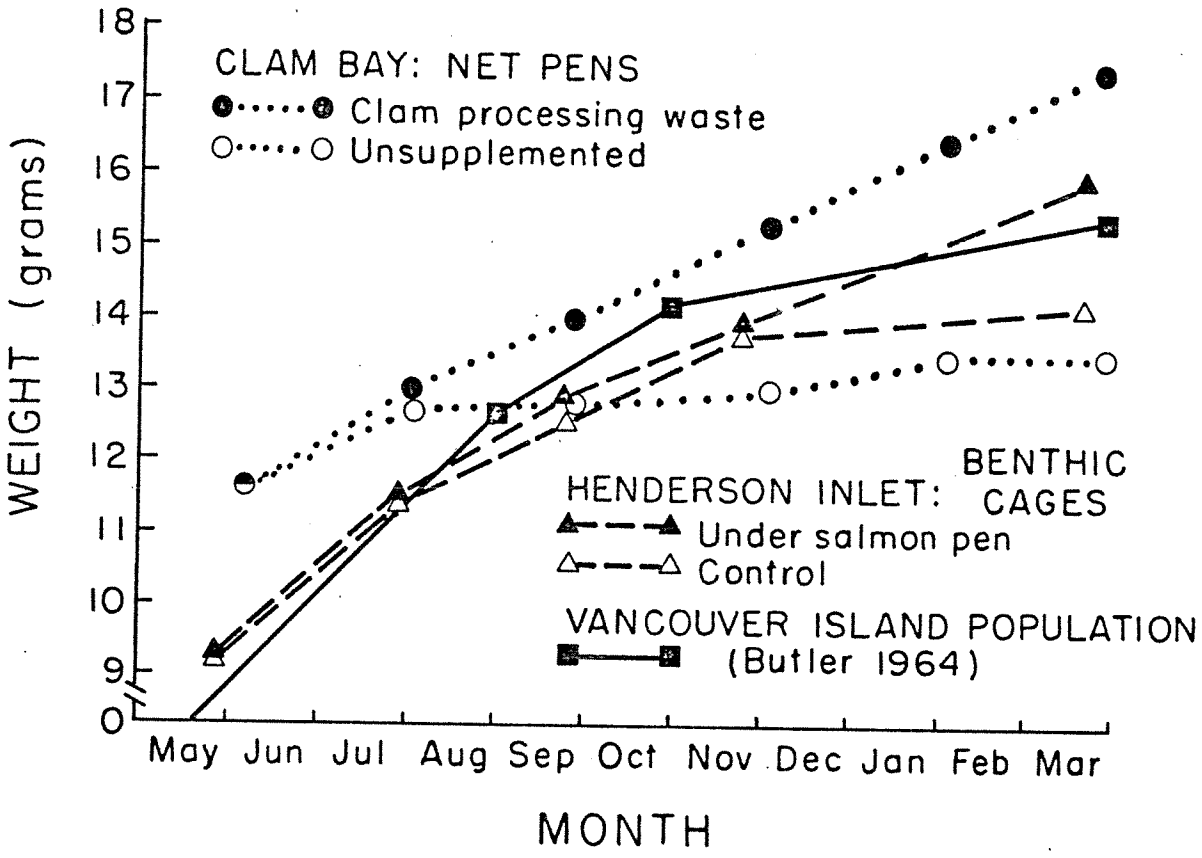

Fig. 18. Mean weights of yearling prawns reared at Clam Bay and Henderson Inlet, Washington, compared to a British Columbia population (Butler, 1964).

Table 6. Average carapace lengths and weights, ranges, standard deviation, interval and specific growth rates of yearling prawns reared in net-pens at Clam Bay, Washington from June 1974 to March 1975

sampling date & identification	average carapace length (mm)	standard deviation	carapace length range (mm)	average weight (g)	standard deviation	weight range (g)	specific growth rate (g/g/day)	interval weight increase per day
5 June 1974								
Clam Fed	26.0	1.68	20.0-29.0	11.6	2.39	4.8-16.4	-	-
Unsupplemented	26.2	1.68	21.0-29.0	11.6	2.12	5.4-15.5	-	-
2 August								
Clam Fed	26.8	1.72	21.0-30.0	13.0	2.36	6.5-17.5	.0020	.024
Unsupplemented	26.8	1.53	21.0-30.0	12.7	1.53	6.8-17.0	.0016	.019
27 September								
Clam Fed	27.5	1.58	24.0-30.5	14.0	2.14	7.3-18.8	.0013	.018
Unsupplemented	27.1	1.35	23.0-30.0	12.8	1.66	8.9-17.0	.0003	.003
2 December								
Clam Fed	28.7	1.45	25.0-33.5	15.3	2.10	9.0-19.8	.0014	.021
Unsupplemented	27.7	1.24	24.0-31.0	13.0	1.58	9.5-16.2	.0002	.002
2 February 1975								
Clam Fed	29.2	1.47	24.5-33.5	16.4	2.09	11.4-22.4	.0011	.018
Unsupplemented	27.8	1.25	25.0-31.0	13.4	1.62	9.2-17.1	.0005	.006
28 March								
Clam Fed	29.8	1.44	25.5-35.0	17.1	2.21	12.3-26.8	.0010	.017
Unsupplemented	28.0	1.23	24.5-31.0	13.4	1.60	9.5-17.3	.0000	.000

(brown filamentous forms), diatoms (particularly Riddulphia sp.) and tiny sections of exoskeleton from either the prawn's exuvia or naturally occurring crustaceans in the net pens.

Net fouling organisms were preyed upon by prawns in both treatment groups. Commonly seen net fouling organisms (mussels, tunicates, bryozoans) were noticeably absent from the nets after 10 months of immersion. The only major fouling organisms present were hydroids (Obelia sp.) and entoprocts (both restricted to the top 10 cm of the net).

Yearlings - Henderson Inlet - Benthic Caged

Control and experimental groups beneath the salmon-pen grew at similar rates ($0.25 < P$) from initiation in late May to late November 1974. (Figs. 17, 18, and Table 7.) Under salmon-pen prawns showed a greater drop in growth rates in the October to November interval (Table 7) which may have been caused by degraded environmental conditions in the sediments and adjacent waters at that time (see survival section). In the final, winter interval rates of growth for the control-prawns greatly declined while those of the experimental group increased so that they were significantly larger ($0.001 < P <$ for length, $0.0001 < P$ for weight).

Yearling Growth Compared with that of a Natural Population

Analysis of covariance for all yearling groups and a reported natural population (Appendix 7) show similar length/weight relationships in that the slopes of the lines are essentially the same.

Table 7. Average carapace lengths and weights, ranges, standard deviation, interval and specific growth rates of yearling prawns reared in benthic cages at Henderson Inlet from May 1974 to March 1975

sampling date & identification	average carapace length (mm)	standard deviation	carapace length range (mm)	average weight (g)	standard deviation	weight range (g)	specific growth rate (g/g/day)	interval weight increase per day
28 May 1974								
Experimental	24.6	1.64	19.5-28.0	9.3	1.71	4.8-12.8	-	-
Control	24.6	1.82	19.5-27.5	9.4	1.80	4.8-12.8	-	-
28 July								
Experimental	25.9	1.26	23.0-28.0	11.5	1.55	7.8-14.8	.0035	.036
Control	25.9	1.30	22.0-28.0	11.4	1.55	6.7-14.4	.0031	.032
25 September								
Experimental	26.9	0.99	24.5-29.5	12.9	1.24	9.8-16.1	.0020	.024
Control	27.0	1.02	23.5-29.0	12.6	1.20	8.5-15.4	.0017	.020
24 November								
Experimental	27.5	0.70	26.0-29.0	13.9	0.80	12.4-15.7	.0011	.014
Control	27.7	1.01	25.0-29.5	13.8	1.42	9.6-15.8	.0015	.018
21 March 1975								
Experimental	29.1	0.99	26.5-30.5	15.9	1.21	12.6-18.22	.0012	.017
Control	28.4	1.16	25.0-31.0	14.2	1.71	9.6-18.55	.0002	.005

The length versus time relationships (Appendix 8) show significant differences between the following three groups listed in order of decreasing slope (and average growth rate): 1. prawns in benthic-cages beneath salmon pens, 2. prawns fed clam processing wasted in net pens and the benthic cage control, 3. prawns of the reported natural population (Butler, 1964) and unsupplemented prawns in net pens.

Weight versus time relationships (Appendix 9) follow the same order but each treatment was significantly different from the others (all five were statistically different). Thus in terms of rate of weight gain all treatments except the net pen reared, unsupplemented yearlings grew at higher rates than the reported British Columbia population.

Molting

Yearlings - Clam Bay Surface Pens

There were five major molting peaks during this study. A higher percent of clam fed prawns molted at each peak than did unsupplemented prawns but periods of peak molting coincided for each group (Fig. 19). Two major molting peaks occur in summer about 50 days apart. Seventy-five days later a peak occurs which in turn is followed by another peak in 75 days (Feb. 1). The final peak occurs after a 50 day interval (March 20) at which time the experiment was terminated. A pattern emerges as molting peaks occur at either 1.6 or 2.5 month intervals.

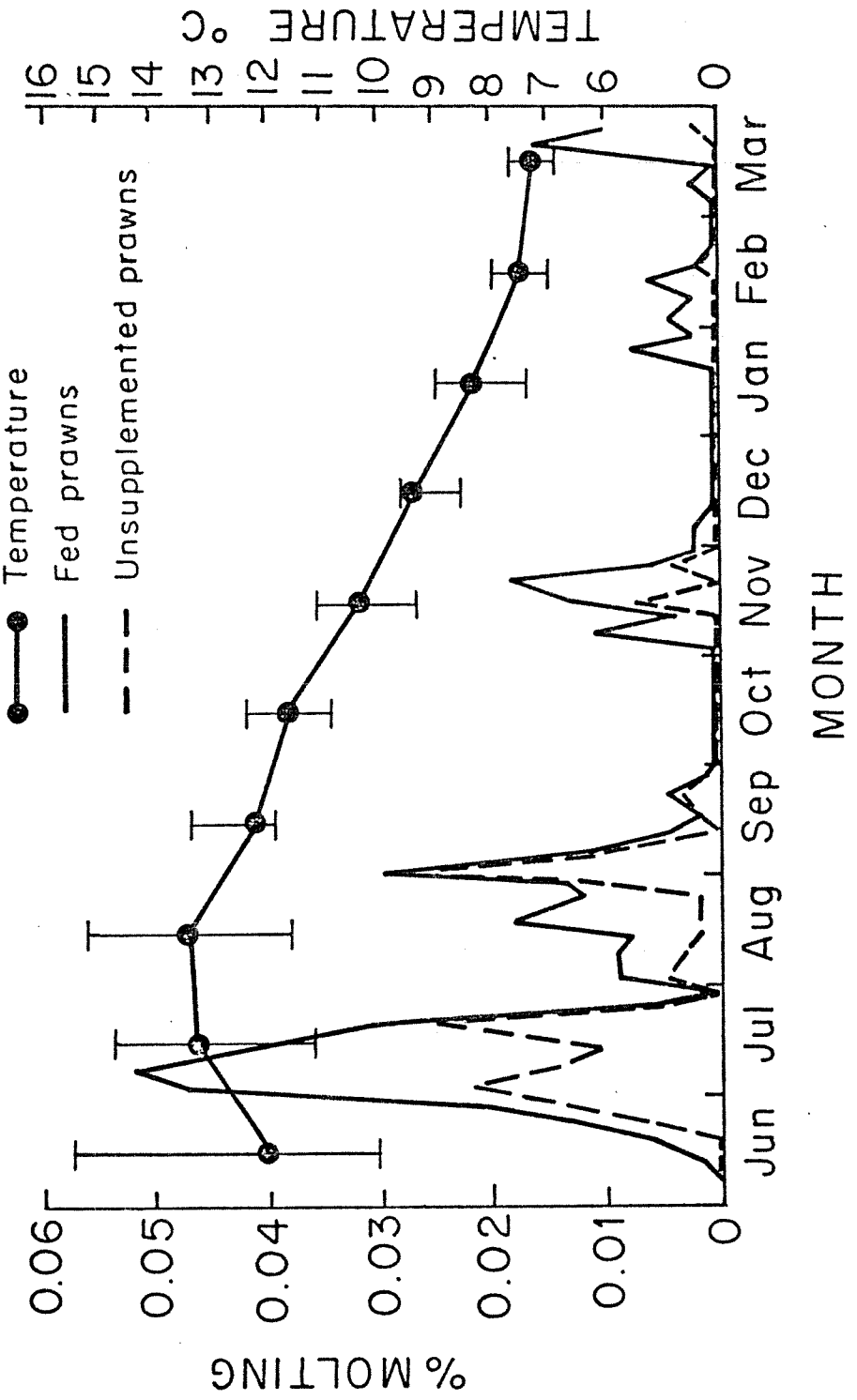

Fig. 19. Five day mean percent molting of clam processing waste fed and unsupplemented yearlings compared to mean and range of 0.5 m water temperature at Clam Bay, June 1974 to March 1975.

DISCUSSION

Survival

Juveniles

Excessive handling and lack of an acclimation period could explain the initial losses at Henderson Inlet. In addition to periodic measurement at both sites, juveniles at Henderson Inlet were further handled three times weekly since limited water visibility necessitated pulling the net-pens to the surface to remove old food or mortalities. Lightner and Lewis (1975) have found that cuticular injuries from handling of penaoid shrimp may result in bacterial septicemic diseases particularly Vibrio sp. Presumptive confirmation that Vibrio sp. was present in prawn hemolymph was obtained by microscopic examination of wet mount slides from plate cultures of moribund prawns at Henderson Inlet. High total counts of bacteria in surface waters during summer months (Westley et al., 1973) coupled with handling-caused cuticular breaks of recently molted juvenile prawns was the probable route of infection.

The above mentioned mortality followed by further losses from a dense plankton bloom in early September 1974 resulted in a final survival of only 30.3 percent.

Survival was much better at Clam Bay (79.3 percent in March 1975) but there seemed to be a continuous low level of mortality throughout the experiment. I was unable to detect the cause(s) of these losses although cannibalism and accidental discarding of juveniles with mussel shells are possibilities.

Yearlings - Henderson Inlet - Surface Pens

Studies by the Washington Department of Fisheries (Westley et al., 1973; Tim Schink, personal communication) in southern Puget Sound inlets have noted summer time water quality problems. Results of oyster and clam larvae bioassays indicated "questionable" water quality in nearby Budd Inlet; samples from other inlets frequently had similar results. Multiple regression analysis of environmental data did not reveal the responsible factor(s). However phytoplankton blooms, bacteria and their toxins have been implicated as possible sources of the seasonal problems that primarily occur in surface waters. Since the epizootic was effectively stopped in prawns that were lowered to the bottom the surface waters may have induced the mortality in some manner. There are four possible explanations that alone or together may explain the mortality. 1) Fouling organisms prevented proper respiratory exchange to take place. This seems unlikely since dissolved oxygen levels (8-10 ppm) were not even near critical (5 ppm) and the gills were generally free of most fouling organisms. The prawns normal gill cleaning appendage, the posterior portion of the second maxilliped (gill bailer) seemed to be effective in keeping most macro-organisms from growing on the gill surfaces although not between the gill laminae. However, prawns relieved of fouling organisms in a formalin dip seemed to recover for a few weeks. 2) The natural route of bacterial infection in shrimp is thought to be through cuticular breaks (Lightner and Lewis, 1975) and since the yearling prawns did not exhibit signs of this phenomena (as juveniles at Henderson Inlet did) it is not likely that bacterial septicemias were prevalent. The feeding of fish food with antibiotics did not have any noticeable

effect. 3) Plankton blooms, bacterial metabolites and toxins may have contributed to the problem. In the previous year Didier (1974) had monitored bacterial levels in the water, plankton and fouling material at Henderson Inlet. It was found that the total counts of bacteria in the water column remained rather constant throughout the study (summer and fall). Highest levels of bacteria in plankton were found in the beginning of July and appeared to be correlated with a decrease in visibility caused by a plankton bloom. Outbreaks of Vibrio anguillarum in pen reared salmon were related to environmental factors such as temperature extremes rather than to ambient levels of bacteria. In the present study phytoplankton blooms were not particularly intense during the mortality period (June, see Hydrography: light). 4) The final and most plausible explanation for the mortality was the adverse effects of temperature. In early June weekly maximum temperatures increased from 11.8 to 21.9° C in one week while weekly means rose about 5° C (Fig. 3). There is no published temperature tolerance data for adult prawns but juveniles are known to be susceptible to rapid fluctuations and extremes of temperature (Wickins, 1972). Kelly et al. (1976) found the LD_{50} (upper thermal tolerance limit) for larval and juvenile prawns to be 22.9° C, slightly above maximum temperatures encoutered at Henderson Inlet.

Since prawns are found in shallow, relatively warm water until one year of age (Berkeley, 1930), thereafter in deep colder waters; yearling and older prawns may be more sensitive to the effects of temperature extremes and fluctuations than are juveniles. Sixty-five percent survival of juveniles during the July-August period would reinforce this

theory of differential tolerance to temperature extremes. Adult prawns have been held in recirculating systems at high temperatures (7 to 22° C) with little mortality but the temperature increase was gradual, over a period of several months (Price, 1969; K. K. Chew, personal communication).

Yearlings - Clam Bay Net Pens

Survival of yearlings in net pens at Clam Bay was slightly less than in benthic-cage control groups at Henderson Inlet. The clam processing waste fed group was fed every other day but the final survival (78.6 percent) was only 11.9 percent better than the non-supplemented prawns (66.7 percent) that received no direct feeds. This apparent contradiction can be explained as follows. The clam processing waste fed prawns were growing much more rapidly than the unsupplemented prawns throughout the experiment. The higher molt frequency meant that they were vulnerable to cannibalism in the post molt stage more often than the unsupplemented prawns. This cannibalism may be due to the use of a raw, singular source diet. Welsh (1974) has shown that the freshest and most varied diets produced the best survival and feeding response in dungeness crabs (Cancer magister) reared in tidal impoundments. It is probable that the clam processing waste fed prawns in this experiment were subject to a "feeding fatigue" as they were held for 10 months on the same diet. Use of a suitable formulated diet or varied source raw diet would probably improve survival in prawn culture.

Starvation experiments have shown that shrimp (in general) do not store large amounts of reserve material (fats and glycogen) in their bodies (Mistakidis, 1968). Since 60 percent of the unsupplemented

prawns had significant amounts of material in their stomachs at termination these prawns were maintaining themselves on net fouling and/or small pelagic organisms. If salmon and prawns were reared together in the same net-pen the removal of net fouling organisms could be highly beneficial in terms of reduced pen maintenance and cleaning costs, besides providing a varied food supply for the prawns.

Yearlings - Henderson Inlet - Benthic Cages

Control benthic cages had the highest rate of survival of any yearling group (81 percent) in spite of the poor results obtained from surface-pen-prawns at the same site. The mortality that did occur may possibly be related to cannibalism of freshly molted individuals or handling induced stresses.

Survival in benthic-cages under the salmon pens was much lower (46.4 percent) and was a result of adverse environmental factors in the late fall of 1974. Otherwise, survival in this group was similar to the control group in other periods.

In order to eliminate this type of mortality feeding rates of salmon should be followed carefully so that excess feed and enrichment do not pollute the bottom sediments. An effort should be made to use sites that have sufficient depth and flushing capacity.

Implications

The results of these experiments show that Henderson Inlet surface waters were unsuited for prawn culture. A high percentage of the mortalities were related to plankton blooms, protozoan fouling, and fluctuating and extreme surface water temperatures. Benthic-cage

culture at this site is possible but organic enrichment from salmon-pens would have to be limited.

In contrast, surface waters at Clam Bay were more conducive to prawn survival. Juveniles and yearlings (supplemented) had similar levels of survival, about 80 percent.

Effects of Salinity on Growth and Survival

Pandalid shrimp are generally stenohaline in nature and are known to be susceptible to changes in salinity (Panikkar, 1968). A relatively narrow range of salinities was found for yearling and older prawns trawled in British Columbia waters, varying from 26.4°/oo in October to 30.8°/oo in December (Butler, 1964).

Price (1969) used salinities of 29-30°/oo for the culture of larvae and broodstock (older females) in a recirculating water system. The stock originated in Hood Canal, Washington, where salinities rarely rise above 31°/oo. Poor survival was reported for larval culture but adults were held with no difficulty. Wickins (1972) found the effect of salinity to be "more pronounced than the effect of temperature for growth and survival of juvenile prawns." In his experiment juveniles were subjected to salinities of 22, 26 or 30°/oo at temperatures of either 15, 18 or 20° C. The author states that optimum salinity was not less than 30°/oo although no values above this were evaluated experimentally. The lowest salinity (22°/oo) was especially growth inhibiting at a temperature of 20° C which is opposite the effect found for many other shrimps that are of interest as potential aquacultural species (Panikkar, 1968).

Larvae and post larvae demonstrated tolerance to relatively low salinities in short term bioassays performed by Kelly et al. (1975). Only 20 percent mortality occurred at 22°/oo but lower salinities induced a rapid increase in deaths resulting in a LD_{50} of 20.4°/oo.

Salinity values at the Henderson Inlet were above the minimum values at which prawns exist in nature and were always within 1.5°/oo of the optimum minimum established by Wickins (1972). Therefore the effects of salinity were not thought to be limiting to the prawn's growth and survival in experiments at Henderson Inlet.

Although salinities were not monitored at Clam Bay or at Henderson Inlet after December 1974, previous work (Moring, 1973; Barnes and Collias, 1956a, 1956b) has indicated that salinities in both areas rarely drop below 27°/oo. Thus the prawns in my experiments were probably not detrimentally affected by salinity at either of the culture areas.

Effects of Dissolved Oxygen on Growth and Survival

A primary and often critical limiting factor in the production of aquatic animals is the level of dissolved oxygen in culture waters. Bardach et al. (1972) reports that 3.5 ppm is the minimum acceptable level of dissolved oxygen for pond production of the Kumura shrimp, Penaeus japonicus. Rickards (1971) cultured pink shrimp, Penaeus duorarum in laboratory tanks that also contained benthic diatoms and various algal fouling materials. Nighttime dissolved oxygen routinely dropped below 5.0 ppm (minimum 3.5 ppm) with no mortality.

Penaeid shrimp are considered more primitive than shrimps of other suborders such as those of the caridea which include the family pandalidae.

There are, however, many similar morphological features between the two genera (Young, 1959) which implies the existence of similar although more advanced metabolic systems in pandalid shrimp. Therefore, taking into account critical levels mentioned above for penaeid shrimp and the general lower limit of 5 ppm for marine invertebrates in general as presented by Riesch (1970) we may assume dissolved oxygen to become limiting to growth and survival for prawns when levels drop below 5 ppm.

Weekly mean 2.0 m and 1.0 m above bottom dissolved oxygen levels were always 1 ppm above the critical level at Henderson Inlet. On only one occasion, in mid-September at 2.0 m, did recorded minimum values decline to the critical level of 5 ppm. This drop may have been caused by the cessation of the summer's most intense phytoplankton bloom followed in turn by a period of eutrophication. As dissolved oxygen levels declined there was no observable effect on juvenile prawns, the only surface group still being cultured at Henderson Inlet.

Dissolved oxygen was probably critical to the survival of yearling prawns reared in benthic cages beneath a salmon pen. On several occasions in the August-October period values approached the critical level.

Since conditions in the beneath-salmon-pen sediments were somewhat degraded during this period (see survival section), the likelihood of oxygen depleting biochemical oxygen demand (BOD) was high. Dissolved oxygen was measured at 1.0 m above bottom but prawns were always confined to the bottom 0.5 m where dissolved oxygen levels could be seriously depleted during slack tidal periods by the BOD. It is impossible to sort out which factor (low dissolved oxygen, hydrogen sulfide presence, toxic metabolite concentrations) or combination of factors were responsible for the observed mortality.

Plots of dissolved oxygen and growth rate versus time do not show significant trends. The effects of temperature and/or body size (for juveniles) or diet are more pervasive, effectively masking the effects of dissolved oxygen.

Effects of Light on Growth and Survival

Light is an extremely important factor in controlling the behavior of shrimp although its relationship to growth and survival is not clear. Prawn larvae are positively phototaxic in early stages, a feature that allows them to be transported by currents to inshore areas for post larval and juvenile growth phases. Upon assuming a benthic mode post larvae are known to seek cover during the day and become active only at night (Barr, 1973). These traits are important in later life as Chew et al. (1974) have shown in the existence of a definite diel migration of yearling and older prawns in Hood Canal, Washington, to depths of 60 fathoms.

In crustacean aquaculture systems light is important in terms of behavior and survival. As noted above post larval and older prawns actively avoid lighted areas and do so in large net-pens or uncovered ponds and tanks. During the daylight hours prawns are often overcrowded into dark recesses of the culture system which could induce environmental stress, cannibalism of newly molted individuals or difficulty in feed presentation. Older prawns have accumulated algal fouling organisms on the cephalothorax and gills which may have interfered with respiration (Prentice and Rensel, unpublished data). Young prawns, such as juveniles, are not so vulnerable to the effects of light induced fouling as they

molt more frequently. In culture systems the effects of light on prawn growth and survival are not fully understood. Forester (1970) in laboratory culture of Palaemon serratus (another temperate water caridean prawn) reported significantly higher rates of growth for juveniles held in total darkness. Other groups were held in dim light (6 lm/ft^2), natural daylight and bright light (100 lm/ft^2).

In the present experiment extinction coefficients (1.7 ÷ secchi disc reading) did not seem related to growth rates except for juveniles reared in net-pens at Henderson Inlet (Fig. 20). In this case there was an inverse relationship that indicates increases in visibility may be related to increased growth rate at this site.

In all waters surface light levels are attenuated with increased depth due to scattering and absorption of light rays. Naturally, light levels on the bottom at Henderson Inlet appeared greatly reduced which may have been a factor in producing the rapid growth of benthic caged prawns. Surface reared prawns were exposed to large variation in light levels as the pens were uncovered and covered daily for care and inspection. This stimulus coupled with daily human activity nearby could increase catabolic rates and possibly stress conditions.

Effects of Salinity, Dissolved Oxygen and Light on Growth and Survival: Implications

At both sites salinity did not appear to be limiting to growth or survival at any time during this study.

Dissolved oxygen levels at Henderson Inlet were above the critical point at all times. On a few separate days during the fall dissolved oxygen levels approached the critical point (5.0 ppm) in surface and

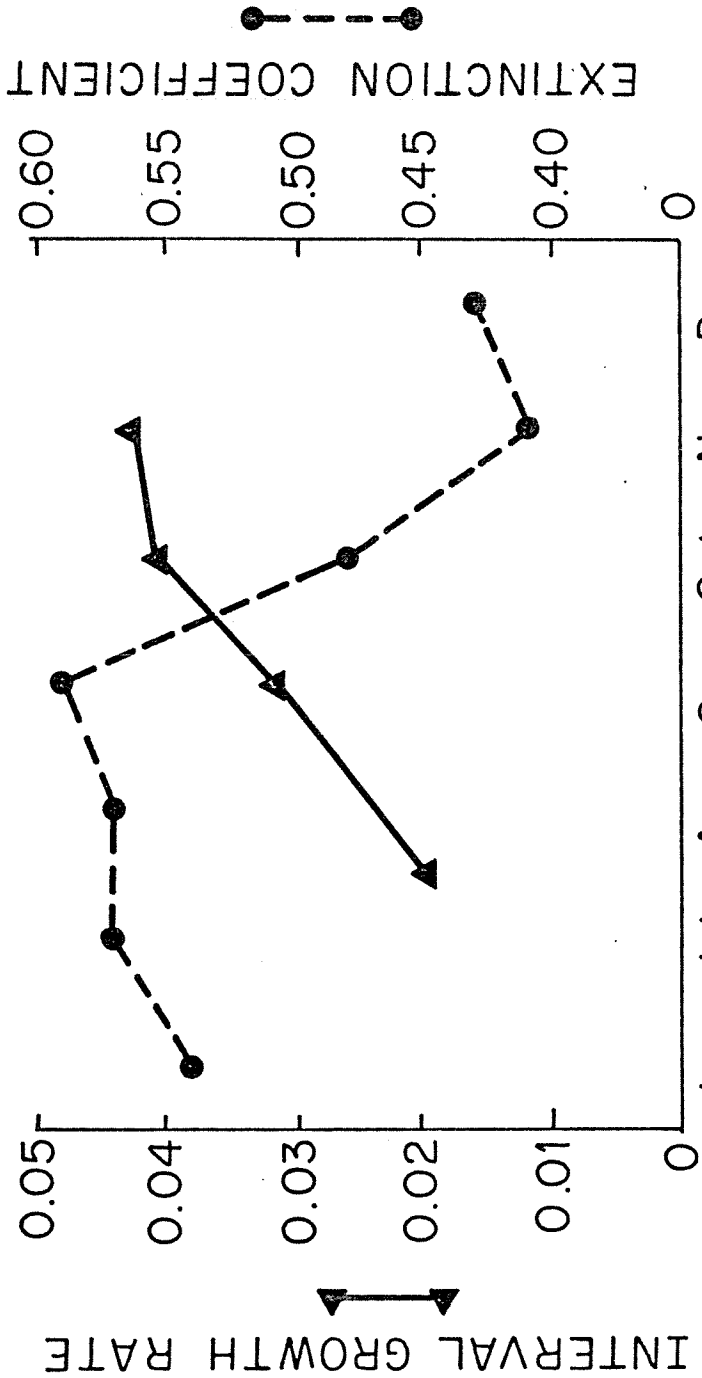

Fig. 20. Interval growth rates of net pen reared juvenile prawns and extinction coefficient versus time for Henderson Inlet, June to December 1974.

bottom waters. Poor survival and reduced rates of growth may have resulted from the effects of the dissolved oxygen depression and other synergistic factors.

Light levels at Henderson Inlet were much lower than at Clam Bay. Increasing light levels in the fall months at this site were correlated with a rist in the growth rate of net pen reared juveniles. There was no consistent trend of mean (secchi disc) visibility at Clam Bay and therefore no relation with growth or survival.

Molting

Yearlings - Clam Bay Surface Pens

Rickards (1971) found a highly significant relationship between temperature and frequency of molting for tank reared, juvenile Penaeus duorarum. In the present study percent of prawns molting appears related to temperature (Fig. 19) but molt frequency is not since it increases in late winter while water temperatures are still depressed. A previous study of juvenile prawns has shown that molt frequency decreases with age (Wickins, 1972); however, there is no published account for molt frequency of older prawns. Kamiguchi (1971, cited by Wickins and Beard, 1974) has reported that molt frequency of sexually immature Palaemon paucidens decrease with size until maturity when constant intervals between molting peaks was the rule.

Since prawns used in the present study were reaching sexual maturity midway through the experiment the molt pattern may have been affected.

Another possible influence is photoperiod. Generally, prawns molted more frequently in summer and early spring than in the fall and

winter. Thus molting activity is roughly correlated with periods of longest photophase.

Effects of Temperature and Treatment on Growth

Juveniles

The expected benefits of increased temperature were not realized in the growth of Henderson Inlet juveniles although 0.5 m water temperatures averaged 2.5° C warmer than Clam Bay waters during summer months.

A plot of mean monthly temperatures versus specific growth rate of the Henderson Inlet juveniles shows a positive correlation between the two (Fig. 21) in which growth rate declines with temperature.

In contrast, when temperature is plotted against interval weight increase an inverse correlation appears, indicating higher rates of growth in late fall when temperatures are decreasing (Fig. 22). Thus although the number of grams increase per grams of body weight declined throughout the experiment, net gain per interval increased.

At Clam Bay both specific and interval growth rates are positively correlated with changes in mean water temperature (Figs. 23 and 24).

To evaluate temperature as an effect in the growth curves presented above (Figs. 15 and 16) mean carapace lengths and weights were plotted against cumulative degree days (sum of daily 0.5 m water temperature) and are displayed in Figs. 25 and 26. If temperature was the main factor in controlling growth than the curves would be similar.

Both Figs. 25 and 26 show a marked difference in growth suggesting the entrance of other important factor(s). Significantly, the slope of both lines in each figure from September to December appear similar

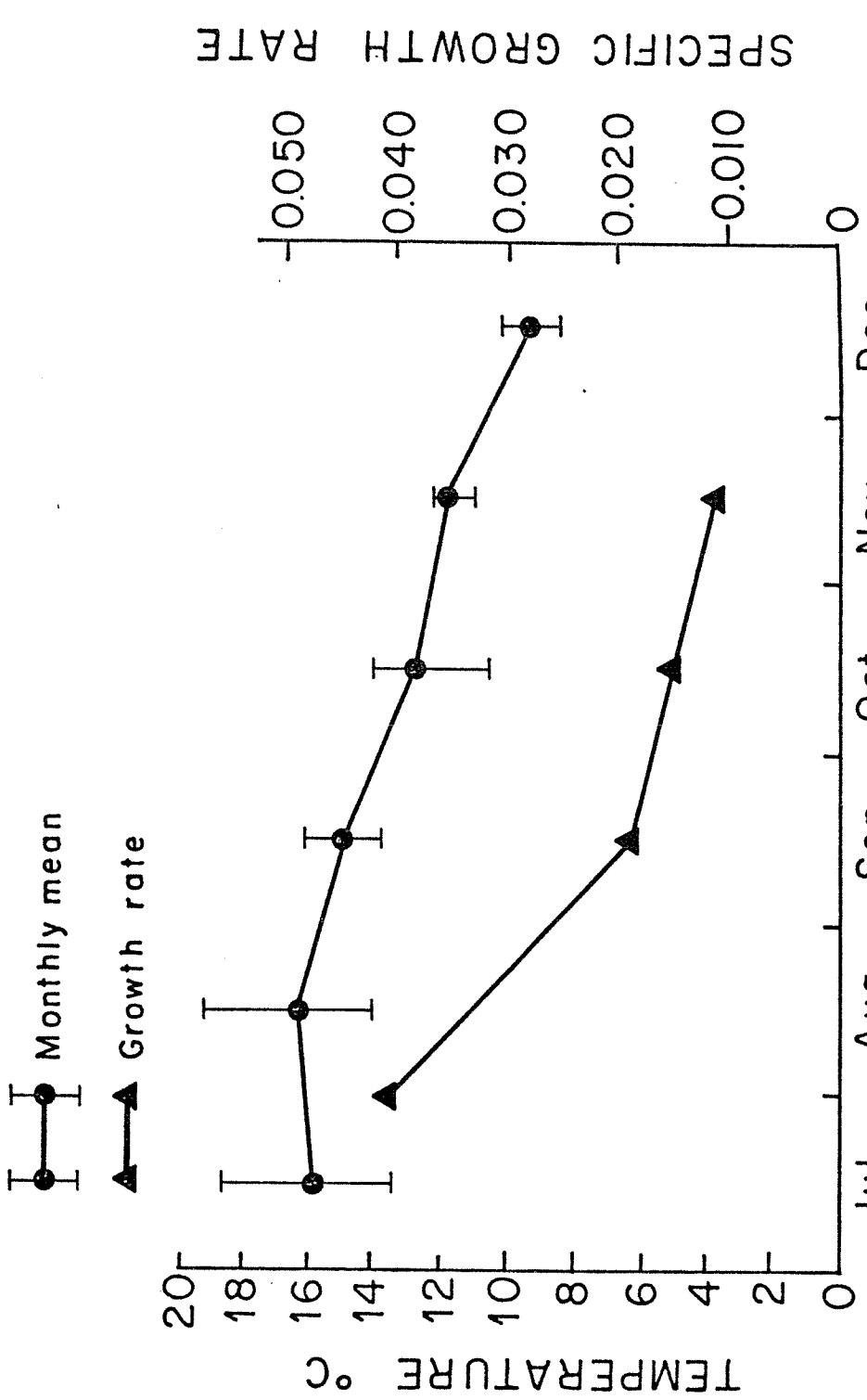

Fig. 21. Monthly mean and range of 0.5 m water temperatures versus specific growth rates of juvenile prawns reared in net-pens and fed mussel at Henderson Inlet, July to December 1974.

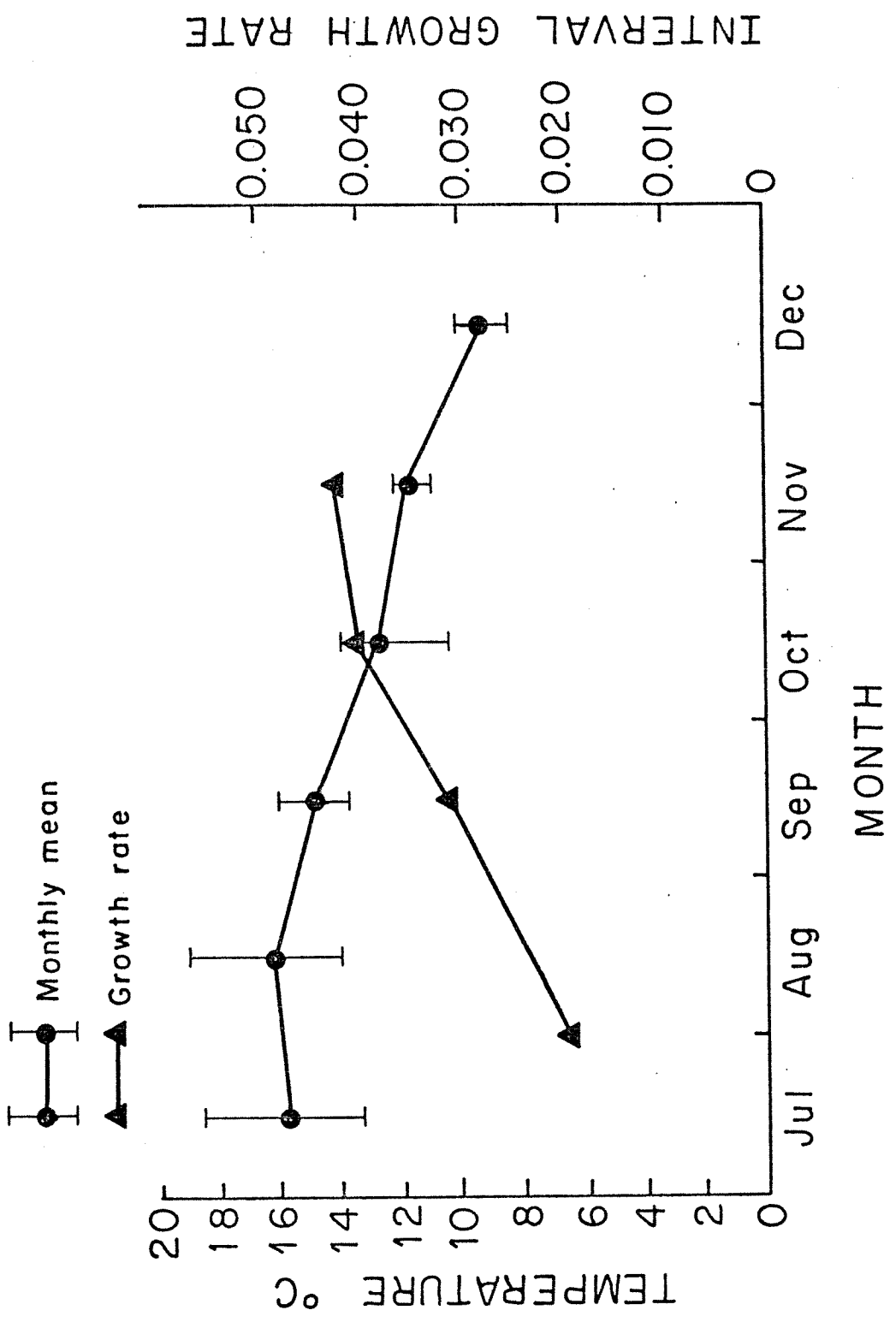

Fig. 22. Monthly mean and range of 0.5 m water temperatures versus interval weight increase of juvenile prawns reared in net-pens and fed mussel at Henderson Inlet, July to December 1974.

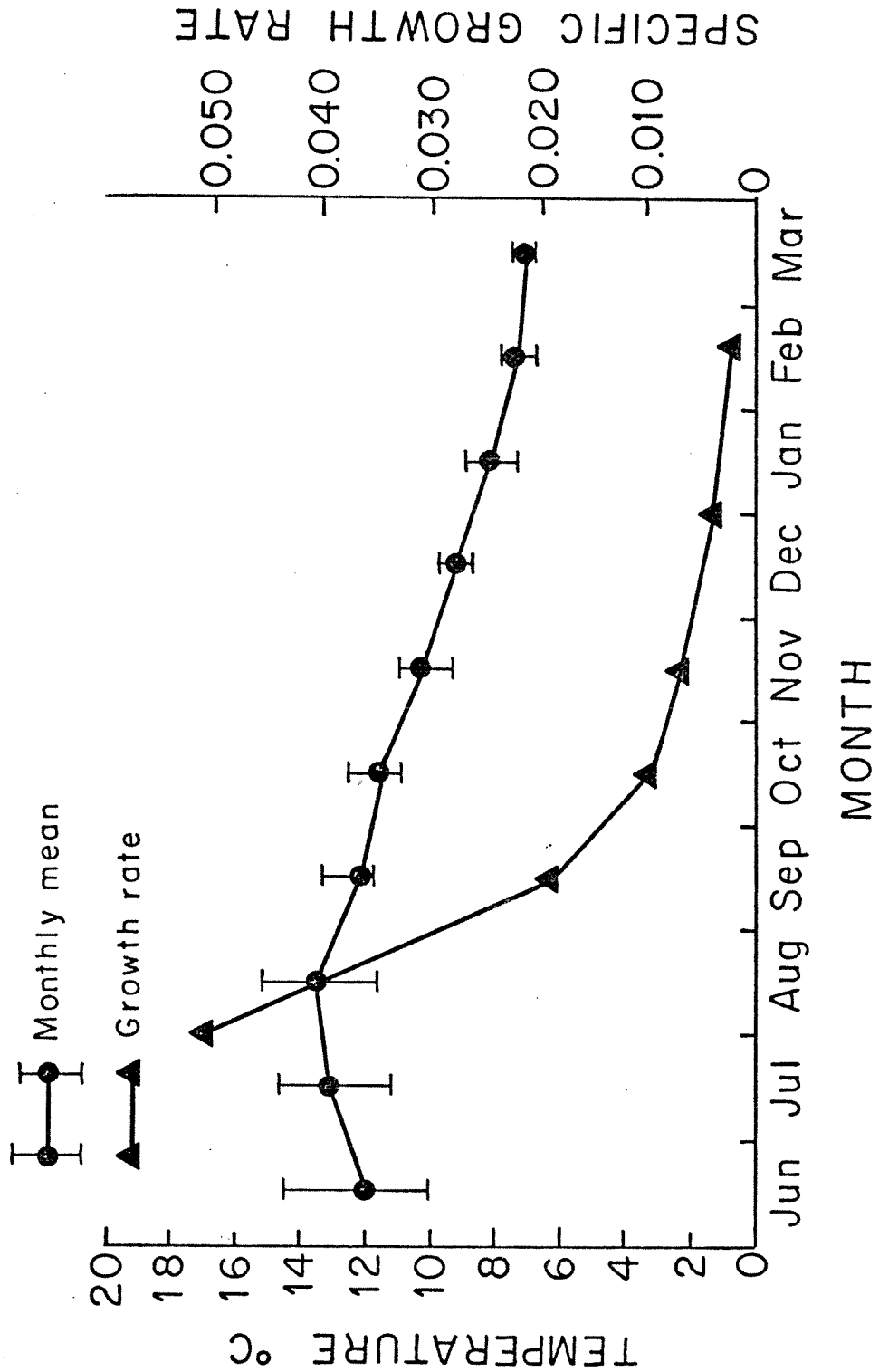

Fig. 23. Monthly mean and range of 0.5 m water temperatures versus specific growth rate of juvenile prawns reared in net-pens, and fed mussel at Clam Bay from July 1974 to March 1975.

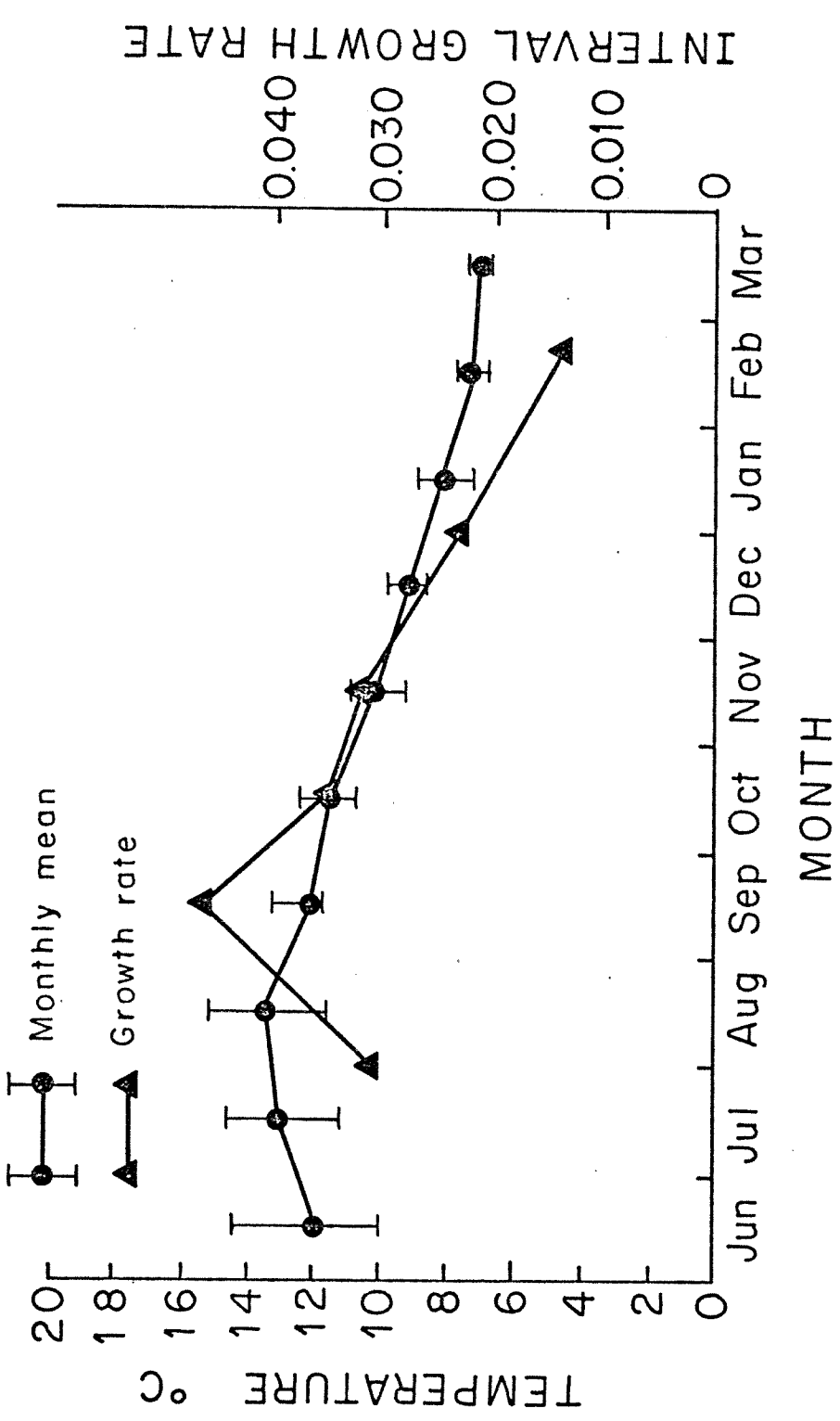

Fig. 24. Monthly mean and range of 0.5 m water temperature versus interval weight increase of juvenile prawns reared in net-pens and fed mussel at Clam Bay, from July 1974 to March 1975.

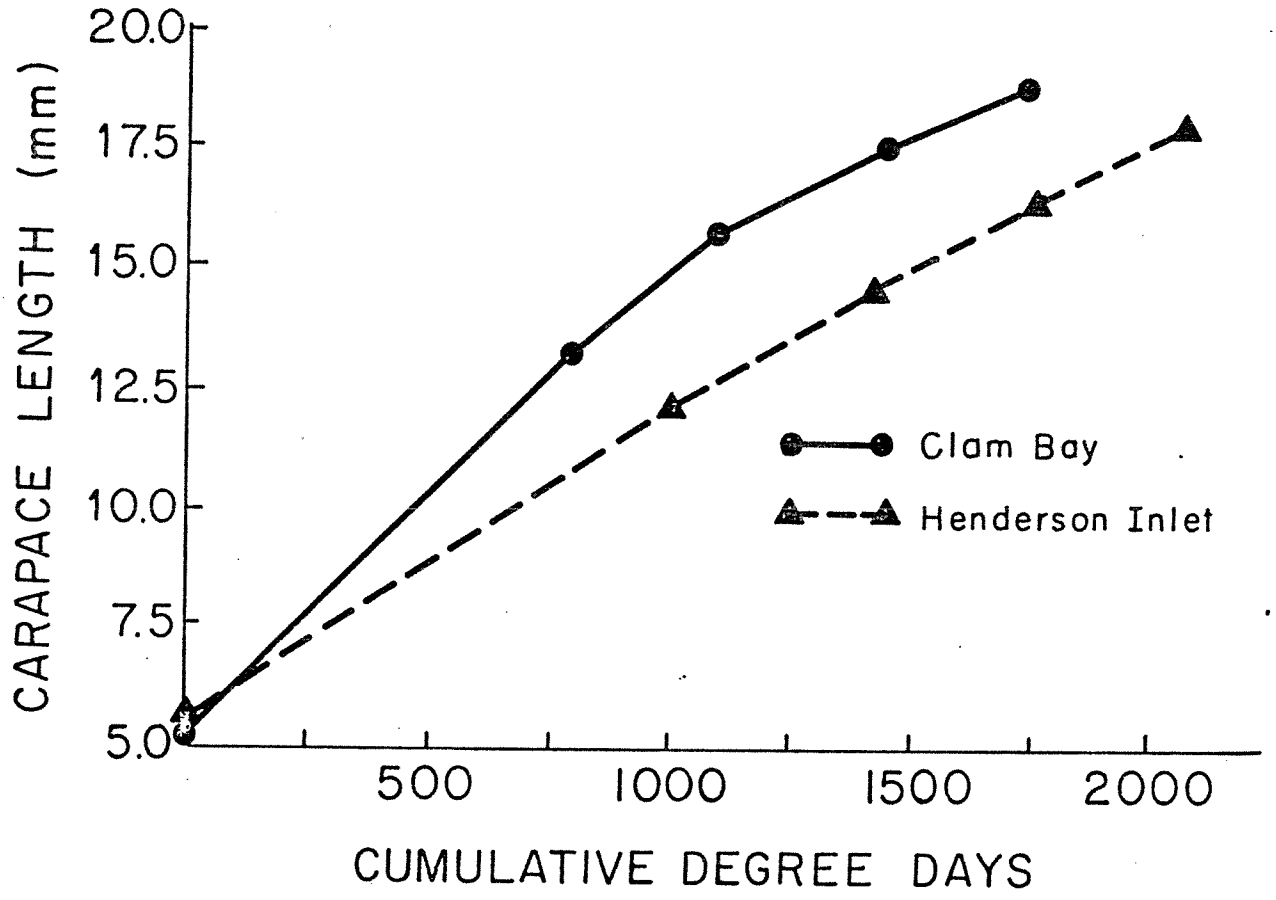

Fig. 25. Growth curves of juvenile prawns plotting average carapace length versus cumulative degree days, from July to December 1974.

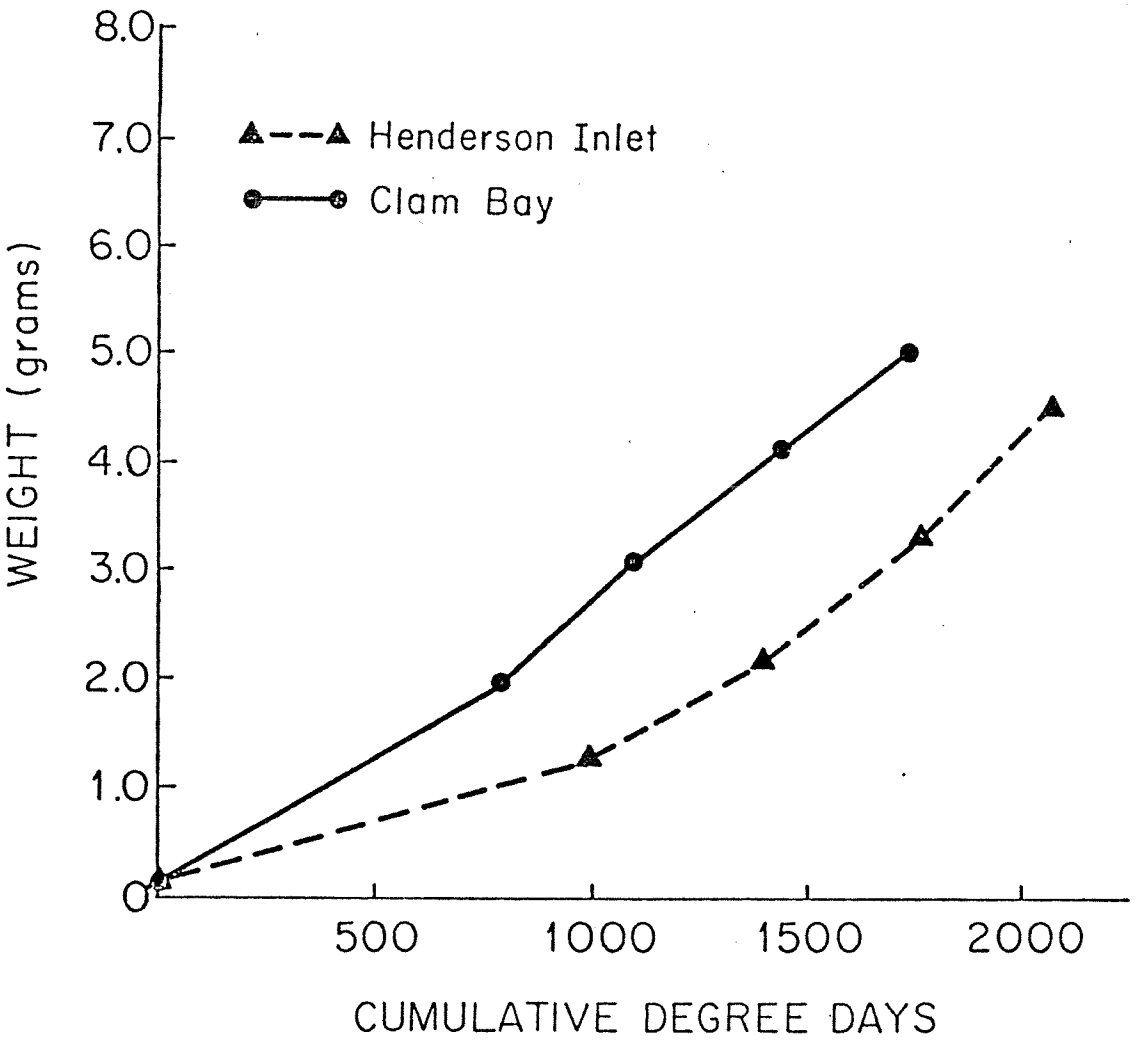

Fig. 26. Growth curve of juvenile prawns plotting average weight versus cumulative degree days, July to December 1974.

indicating that temperature is a prime factor in controlling growth during this period at both sites. Water temperatures encountered at Henderson Inlet in this experiment (July to December 1974 in Fig. 3) seemed near ideal for juvenile prawn culture. Wickins (1972) has found 18° C to be the optimum temperature for juvenile growth and survival. Henderson Inlet waters only exceeded this value by a degree, the mean temperature averaged but a few degrees below the optimum (Fig. 3).

It is interesting to note that maximum interval weight increase occurs at both sites when the monthly mean temperature is near 12°C (Figs. 22 and 26). Factors contributing to the growth depression at Henderson Inlet in summer and to the apparent inverse correlation of water temperature and growth rate (Fig. 22) mentioned above were the most likely the same influences that caused considerable mortality. Intense plankton blooms and frequent handling are probable causes (see survival section).

Yearlings - Clam Bay Surface Pens

The clam processing fed prawns grew in a linear like fashion, in terms of length and particularly weight (Fig. 17 and 18). Plots of specific and interval growth rates of unsupplemented and clam fed prawns versus time (Figs. 27 and 28) show no correlation with temperature. Clam fed groups had growth rates that were constant or slightly decreasing over a period of 10 months; a period that encompassed a wide range of water temperatures. These prawns seemed temperature independent but perhaps diet dependent in their overall growth patterns.

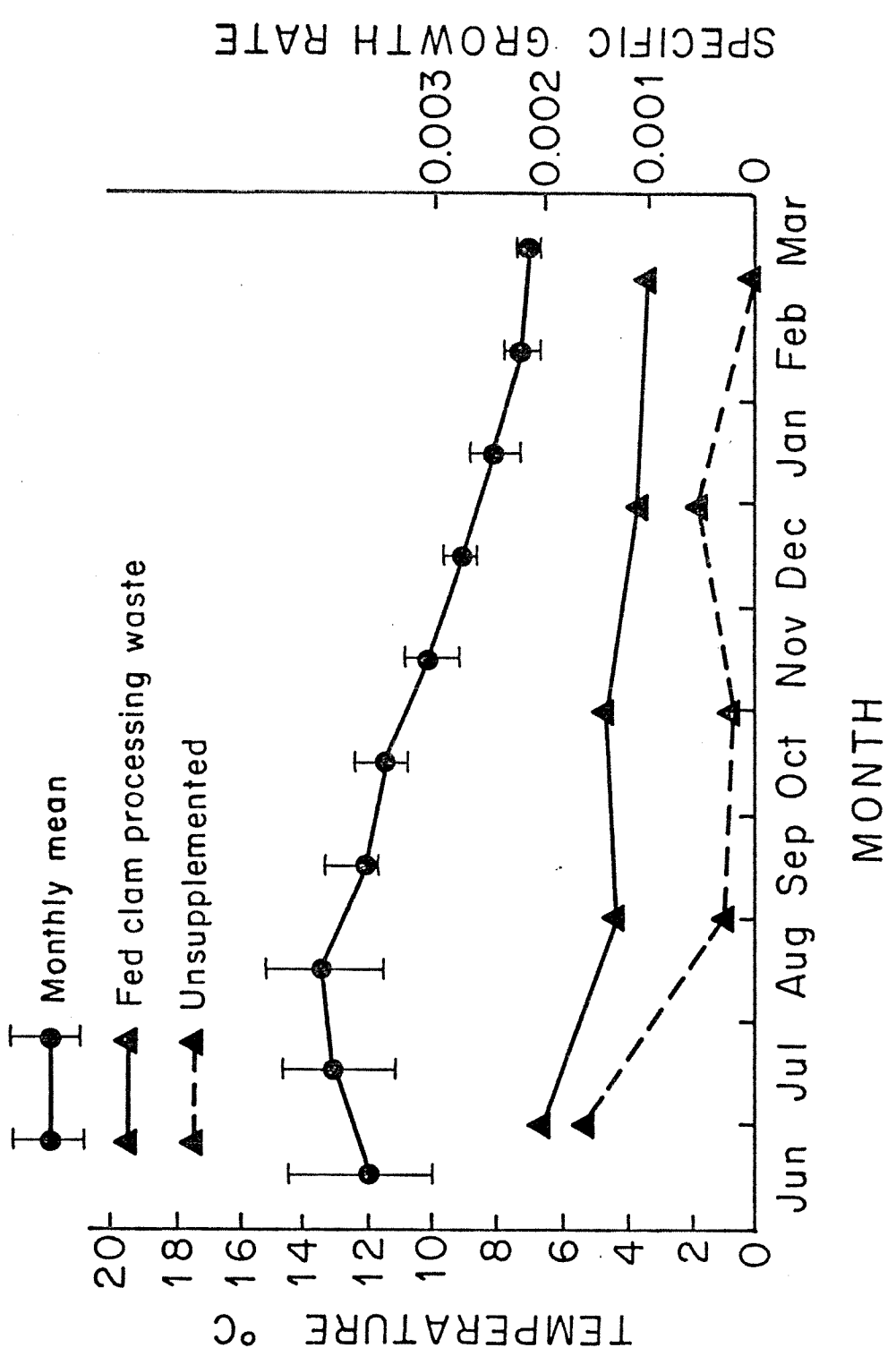

Fig. 27. Monthly mean and range of 0.5 m water temperature versus specific growth rate of yearling prawns reared in net-pens and fed clam processing wastes or unsupplemented at Clam Bay, June 1974 to March 1975.

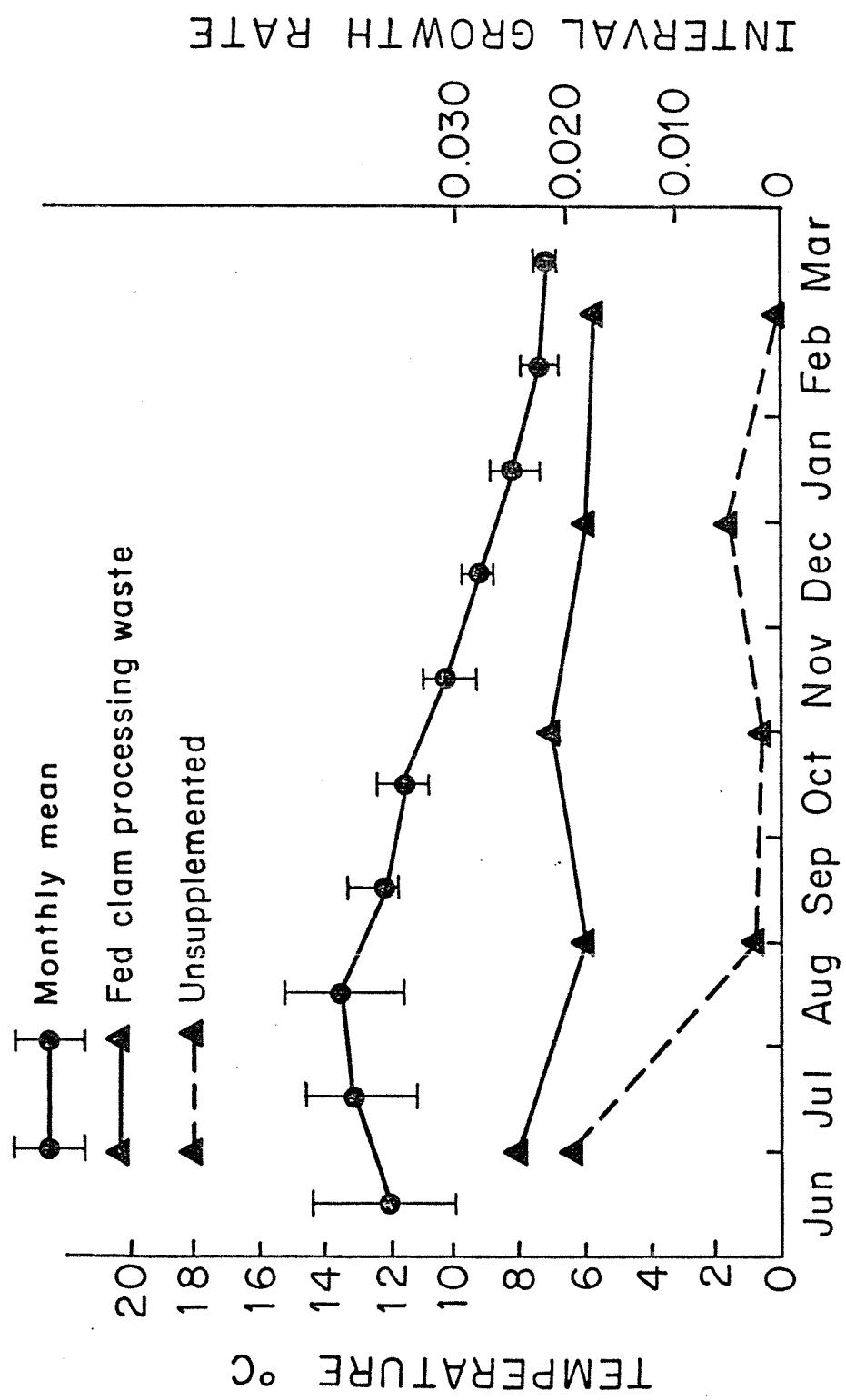

Fig. 28. Monthly mean and range of 0.5 m water temperature versus interval growth rate of yearling prawns reared in net-pens and fed clam processing wastes at Clam Bay, June 1974 to March 1975.

Yearlings - Henderson Inlet - Benthic Caged

Rates of growth for benthic caged prawns generally declined throughout the experiment and do not seem directly related to temperature (Figs. 29 and 30). While there were no temperature records for the January to March 1975 period other studies (Collias et al., 1962, 1974) indicate a pattern of continued decline in water temperatures during this time. In spite of this, growth rates of under salmon-pen prawns increased slightly during the final interval.

There are several possible explanations for the observed decline of growth rates over the entire experiment. Larval recruitment of benthic fauna occurs during spring and summer months. During these periods prawn growth was relatively greater than in the fall and winter when recruitment and the effects of predation may have reduced the available food supply. Mayer (1973) found that the density of Dungeness crabs (Cancer magister) stocked in benthic-cages was inversely proportional to the mean number of invertebrate individuals in core samples from inside the cages. He felt that hydroids and attached carnivores that fouled the cage walls might have acted as a biological filter effectively blocking the transport of pelagic larvae into the pen. In the present study cage fouling may have reduced recruitment as barnacles set on cages of both treatment groups. Large numbers of the polychaete worm Capitella capitata were present on the walls of cages held under salmon-pens in the fall season only. Woodin (1972) found that polychaete worms growing on the screen (3 mm) of an intertidal cage virtually eliminated recruitment within. Mesh size used in the present experiment was larger

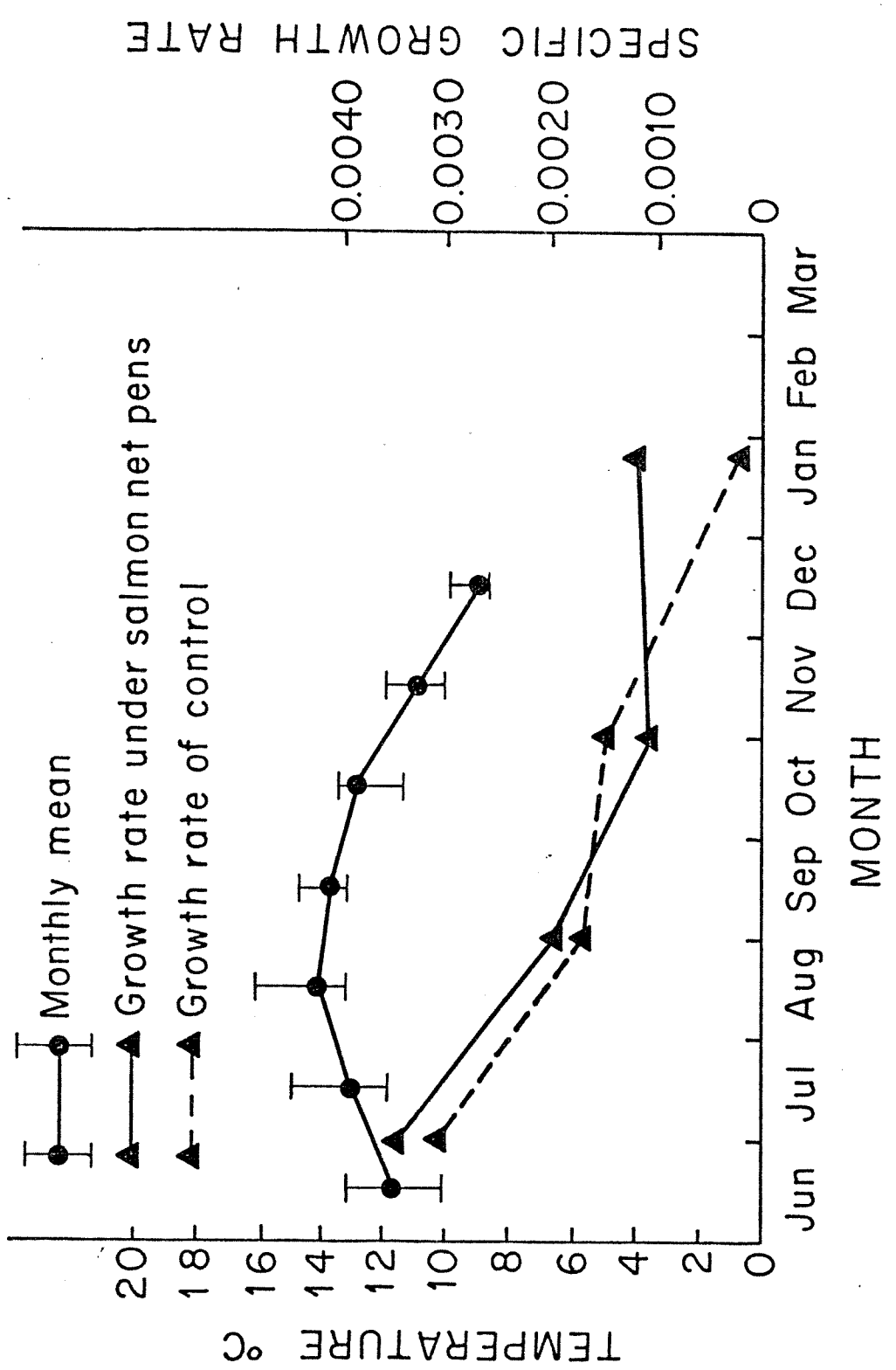

Fig. 29. Monthly mean and range of 1.0 m above bottom water temperatures and specific growth rate versus time for yearling prawns reared in benthic-cages at Henderson Inlet, May 1974 to March 1975.

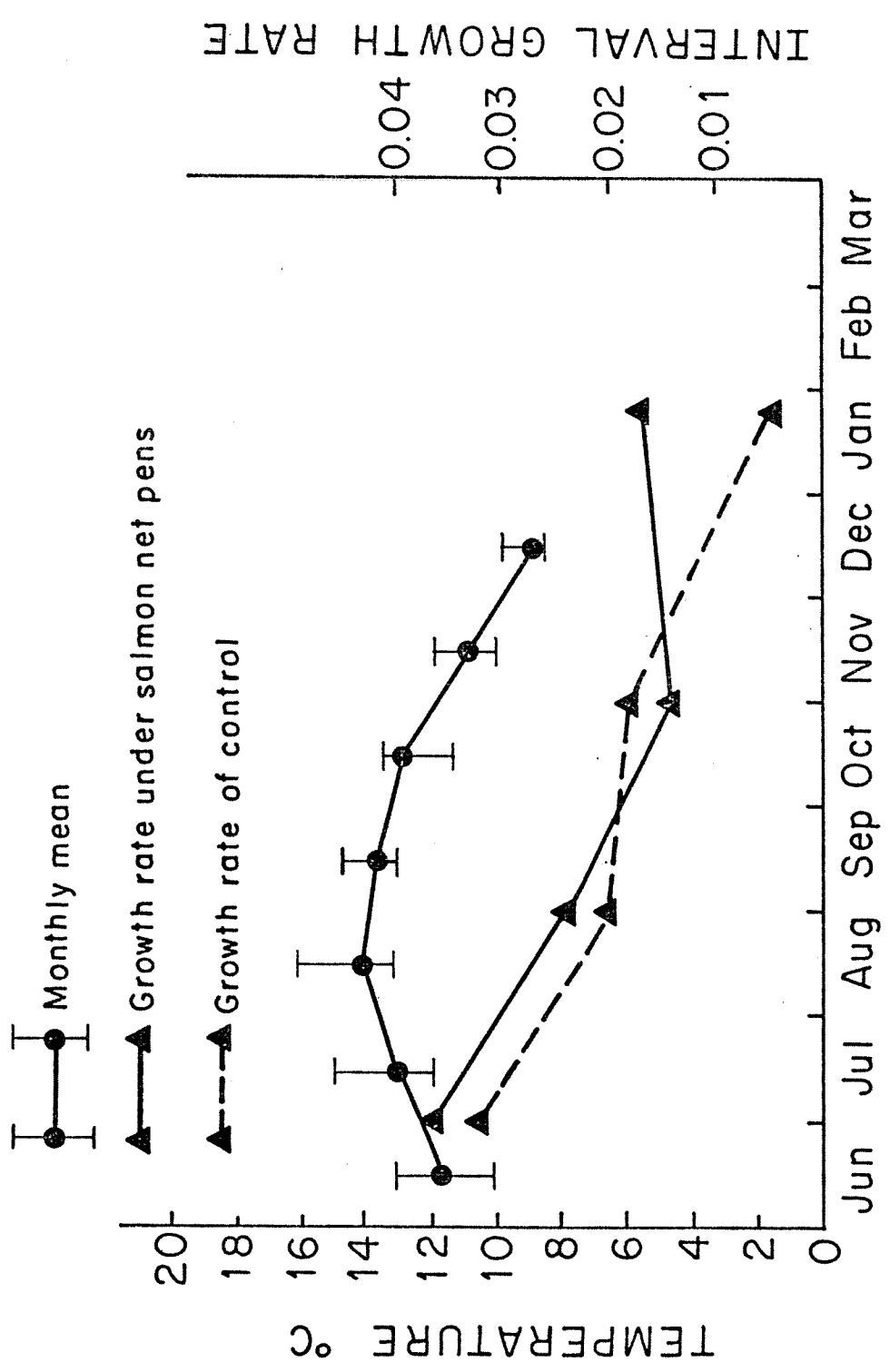

Fig. 30. Monthly mean and range of 1.0 m above bottom water temperatures and interval growth rate versus time for yearling prawns reared in benthic-cages at Henderson Inlet, May 1974 to March 1975.

(13 mm) and, except for the October-November period under the salmon-pens, did not have the heavy fouling that was reported in the two previously mentioned experiments.

Implications

Temperature. The results of this study demonstrate that the growth of juvenile prawns was positively correlated with water temperature. However, there was no apparent relationship for yearling prawns (fed clam processing wastes). This pattern is similar to that observed by Brett (1969) in sockeye salmon (Oncorhynchus nerka) where growth of young fish is more temperature dependent at a small size becoming increasingly size dependent as they grow.

Accelerated growth of juvenile prawns is possible through the use of heated water (Prentice, 1975) but the costs incurred may be prohibitive on a commercial scale. Increased water temperature in a recirculating system (Tang, 1974) or at a naturally warmer site (as in this study) have promoted the growth of pathogenic organisms or stress conditions that resulted in poor growth and survival of prawns.

Correspondingly, commercial culture of prawns may have to be restricted to areas environmentally similar to the natural habitat.

Treatment. Prawns reared in benthic cages under salmon-pens grew only slightly faster than those fed clam processing wastes in net-pens. However, costs and benefits of the systems are very different. Net-pen systems allow regular inspection and easy manipulation of the prawns but a feed ration is needed to insure substantial growth. Benthic cage culture does not allow for daily inspection but no supplemental feed is required if stocking density is similar to that used in this study.

Unless a natural benthic impoundment is available benthic culture of prawns does not appear to be feasible. Commercial benthic culture is unlikely since it may a) require expensive impoundment facilities, b) be susceptible to damage from currents and debris, c) require the use of expensive diving operations. While not feasible for commercial production benthic cage culture of prawns could be used by government or industry as an in situ bioassay of organic pollution by salmonid net pen farms.

The use of animal processing wastes as a feed for net-pen reared prawns is technically feasible but an inexpensive, reliable supply is difficult to obtain. As an alternative feed source Prentice and Rensel (unpublished data) have fed salmon mortalities from net-pen systems to prawns of all ages. During the summer and fall months substantial quantities (up to 10 percent of the salmon stocked initially) are available for this purpose since they are otherwise buried or discarded.

Although there was no growth data collected concerning salmon/prawn polyculture in this study, subsequent experiments (Rensel and Prentice, unpublished data) have shown considerable potential for this type of approach. Mean weight of polyculture reared juvenile prawns (without supplemental feeding) was 30 percent greater than control prawns fed either mussel or fish diets. There was no evidence of inter-specific predation between the zero-aged coho salmon and the relatively smaller juvenile prawns. Salmon that died due to disease or other factors were readily consumed by the prawns without disease transmission.

SUMMARY

1. Salmon net-pen sites in Clam Bay and Henderson Inlet in the central and southern basins of Puget Sound respectively were compared as potential sites for culture of the spot prawn (Pandalus platyceros). Initially, seasonally warmer waters of Henderson Inlet were hypothesized to accelerate growth.

2. During the 1974-75 seasons juvenile and yearling prawns were:
   a. reared in net-pens (floating pens of nylon mesh netting) with and without salmon
   b. fed regularly one of several rations or simply allowed to feed on net fouling organisms (unsupplemented)
   c. reared in benthic-cages beneath salmon pens and in a control area.

   An effort was made to reduce or eliminate crowding and food availability factors so that the abiotic (environmental) influences could be assessed.

3. Survival was near 80 percent after 10 months for both juveniles and yearling prawns at Clam Bay in net-pens. Unsupplemented yearlings has 67 percent survival at the same site. Results of stomach analysis suggests these prawns maintained themselves on net fouling and small pelagic organisms.

   Surface waters at Henderson Inlet were unsuitable for prawn culture due to extreme temperatures (21.9° C in early June), temperature fluctuations, protozoan fouling and dense plankton blooms. During the summer surface-reared juveniles and yearlings experienced 70 percent and 100 percent mortality respectively.

At Henderson Inlet benthic-caged yearlings in the control area had 81 percent survival while those reared beneath salmon pens had 46 percent survival. Mortality in the latter was attributed to adverse environmental conditions in the fall months. Low dissolved oxygen levels, hydrogen sulfide production and excess organic enrichment from the salmon pens were the most obvious contributing factors.

4. Growth of juveniles at Clam Bay was greater than those at Henderson Inlet where any benefits of increased water temperature were offset by stress conditions responsible for the higher mortality. The Clam Bay juveniles were significantly heavier than a natural population reported by Butler (1964).

Maximum yearling growth rates were observed in benthic cages beneath salmon pens, followed in decreasing order by prawns fed a) clam processing wastes in net-pens, b) reared in control benthic-cages, c) the natural populations and d) prawns in net-pens without supplemental rations.

5. Growth rates of juvenile prawns were directly correlated with water temperature. Yearling prawns fed clam processing wastes in net-pens displayed a steady, temperature independent growth rate within the water temperature range encountered at Clam Bay, i.e., 9 to 14° C.

6. Surface net-pen culture of prawns has more potential for commercial development than does benthic cage culture.

7. Salmon/prawn polyculture (both species within the same net pen) and "feed lotting" of prawns with animal processing wastes are promising

intensive culture systems. Additional research is needed before the economic feasibility of these systems can be fully evaluated. This research should include:

a) development of techniques for rearing young juveniles in high density net-pen systems,

b) improvement of feed delivery and mass handling techniques,

c) monitoring the net cleaning abilities of various sizes of prawns,

d) evaluation of combination, raw diets for elimination of feeding fatigue,

e) studies of growth and survival of prawns and salmon in a pilot polyculture system.

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APPENDICES

Appendix 1. Summary table of analysis of variance results for yearling prawns reared at Clam Bay, Washington

Treatment	Date	Parameter	DF BG	DF WG	Mean Squares	F calculated	F tabular 0.05
Clam P.W.F.	6/5/74	Length	2	333	22.31/3.75	5.94*	3.03
Clam P.W.F.	6/5/74	Weight	2	333	48.72/5.75	8.48*	3.03
Unsupplemented	6/5/74	Length	2	333	24.7/2.84	8.69*	3.03
Unsupplemented	6/5/74	Weight	2	333	36.91/4.53	8.15*	3.03
Between Treatments	6/5/74	Length	1	670	3.95/3.42	1.15	3.86
Between Treatments	6/5/74	Weight	1	670	4.15/3.76	1.10	3.86
Clam P.W.F.	3/28/75	Length	2	256	6.68/2.05	3.26*	3.04
Clam P.W.F.	3/28/75	Weight	2	256	10.19/4.85	2.10	3.04
Unsupplemented	3/28/75	Length	2	216	5.19/1.48	3.50*	3.04
Unsupplemented	3/28/75	Weight	2	216	6.60/2.51	2.63	3.04
Between Treatments	3/28/75	Length	1	476	388.96/1.82	213.12*	3.86
Between Treatments	3/28/75	Weight	1	476	1807.40/3.82	472.95*	3.86

DF = Degree of Freedom
BG = Between Groups
WG = Within Groups
Clam P.W.F. = Clam processing waste fed
* = significant at the 0.05 level

Appendix 2. Summary table of analysis of variance results for yearling prawns reared in benthic cages at Henderson Inlet, Washington

Treatment	Date	Parameter	DF BG	DF WG	Mean Squares	F calculated	F tabular 0.05
Control	5/28/74	Length	3	80	4.47/2.79	1.60	2.72
Control	5/28/74	Weight	3	80	4.63/3.08	1.50	2.72
Experimental	5/28/74	Length	3	80	3.62/3.38	1.07	2.72
Experimental	5/28/74	Weight	3	80	4.36/3.33	1.31	2.72
Between Treatments	5/28/74	Length	1	166	0.10/3.12	0.03	3.90
Between Treatments	5/28/74	Weight	1	166	1.10/3.25	0.34	3.90
Between Treatments	11/24/75	Length	1	85	0.84/1.02	0.80	3.96
Between Treatments	11/24/75	Weight	1	85	0.12/1.85	0.07	3.96
Control	3/21/75	Length	3	64	3.43/1.26	2.72	2.76
Control	3/21/75	Weight	3	64	13.02/2.44	5.33*	2.76
Experimental	3/21/75	Length	3	35	1.42/0.96	1.48	2.87
Experimental	3/21/75	Weight	3	35	1.62/1.46	1.11	2.87
Between Treatments	3/21/75	Length	1	105	12.40/1.23	10.09*	3.94
Between Treatments	3/21/75	Weight	1	105	71.46/2.39	29.86*	3.94

* = significant at the 0.05 level

Appendix 3. Summary table of analysis of variance results for juvenile prawns

Site	Date	Parameter	DF BG	DF WG	Mean Squares	F calculated	F tabular 0.05
Henderson Inlet	7/3/74	Length	2	132	1.62/2.80	0.58	3.06
Henderson Inlet	11/24/74	Length	2	38	1.64/1.50	1.09	3.23
Henderson Inlet	11/24/74	Weight	2	38	0.58/0.80	2.07	3.23
Clam Bay	7/8/74	Length	2	132	4.37/3.15	1.39	3.06
Clam Bay	11/27/74	Length	2	105	4.92/2.04	2.41	3.09
Clam Bay	11/27/74	Weight	2	105	1.66/0.80	2.07	3.09
Between Sites	7/8/74	Length	1	268	7.51/2.97	2.52	3.87
Between Sites	11/27/74	Length	1	147	17.40/1.44	12.11*	3.90
Between Sites	11/27/74	Weight	1	147	8.69/0.76	11.41*	3.90

* = significant at the 0.05 level

Appendix 4. Covariance analysis of the regression of prawn mean carapace length (log y) on mean weight (log x) for Clam Bay juveniles and juveniles of a natural population (Butler, 1964)

Source	dF	Sum x^2	Sum xy	Sum y^2	slope	Deviations from regression		
						dF	Sum of squares	Mean square
Clam Bay	6	184.877	9.087	0.475	0.0491	5	0.0820	0.016
Natural	3	24.427	1.758	0.127	0.0719	2	0.0001	0.00005
Within						7	0.0821	0.117
Regression Coefficient						1	0.0410	0.041
Common	9	209.305	10.845	0.602	0.0518	8	0.1231	0.015
Adjusted means						1	0.0066	0.007
Total	10	220.722	11.147	0.610		9	0.1297	0.014

1. Comparison of slopes F = 0.041/0.117 = 0.35, not significant
   Hypothesis of parallel lines is not rejected (0.25 < P).

2. Comparison of elevations F = .007/.015 = 0.47, not significant
   Hypothesis of equal elevations is not rejected (0.25 < P).

Appendix 5. Covariance analysis of the regression of prawn mean carapace length (y) on time (log x) for Clam Bay juveniles and juveniles of a natural population (Butler, 1964)

Source	dF	Sum x^2	Sum xy	Sum y^2	slope	Deviations from regression		
						dF	Sum of squares	Mean square
Clam Bay	6	4.068	26.486	184.877	6.5098	5	12.4580	2.492
Natural	3	0.281	2.568	24.427	9.1233	2	1.0000	0.500
Within						7	13.4581	1.922
Regression Coefficient						1	1.7980	1.798
Common	9	4.350	29.054	209.305	6.6789	8	15.2562	1.907
Adjusted means						1	0.0445	0.044
Total	10	4.640	30.874	220.722		9	15.3008	1.700

1. Comparison of slopes F = 1.798/1.922 = 0.93, not significant
   Hypothesis of parallel lines is not rejected (0.25 < P).

2. Comparison of elevations F = 0.044/1.907 = 0.023, not significant
   Hypothesis of equal elevations is not rejected (0.25 < P).

Appendix 6. Covariance analysis of the linear regression of prawn mean weight (y) on time (x) for Clam Bay juveniles and juveniles of a natural population (Butler, 1964)

Source	dF	Sum x^2	Sum xy	Sum y^2	slope	Deviations from regression		
						dF	Sum of squares	Mean square
Clam Bay	6	42915.429	1125.797	30.218	0.0262	5	0.6859	0.137
Natural	3	27168.750	491.262	9.514	0.0181	2	0.6307	0.315
Within						7	1.3166	0.188
Regression Coefficient						1	1.1053	1.105
Common	9	70084.179	1617.060	39.733	0.0231	8	2.4219	0.303
Adjusted means						1	1.3238	1.324
Total	10	74370.545	1638.360	39.838	-	9	3.7458	0.416

1. Comparison of slopes F = 1.105/0.188 = 5.88, significant
   Hypothesis of parallel lines is rejected ($0.025 < P < 0.05$)

2. Comparison of elevations F = 1.324/0.303 = 4.37, not significant
   Hypothesis of equal elevations is not rejected ($0.05 < P < 0.10$)

Appendix 7. Covariance analysis of the nonlinear regression of prawn mean carapace length (log y) on mean weight (log x) for benthic caged prawns at Henderson Inlet (under salmon pens or control) and net pen reared prawns at Clam Bay (fed processing wastes or unsupplemented) compared to a reported natural population (Butler, 1964)

Source	dF	Sum x^2	Sum xy	Sum y^2	slope	Deviations from regression dF	Deviations from regression Sum of squares	Deviations from regression Mean square
Clam Bay								
Unsupplemented	5	0.000614	0.001246	0.002819	2.0281	4	2.92×10^{-4}	7.32×10^{-5}
Clam fed	5	0.002637	0.007518	0.021612	2.8514	4	1.74×10^{-4}	4.36×10^{-5}
Henderson Inlet								
Control	4	0.002437	0.007045	0.020687	2.8909	3	3.09×10^{-4}	1.03×10^{-4}
Under salmon pens	4	0.003008	0.009638	0.031373	3.2039	3	4.94×10^{-4}	1.65×10^{-4}
Natural population	3	0.010312	0.030650	0.091098	2.9721	2	1.20×10^{-6}	6.01×10^{-7}
Within						16	0.001273	7.95×10^{-5}
Regression coefficient						4	7.55×10^{-4}	1.89×10^{-4}
Common	21	0.019008	0.056097	0.167578	2.9511	20	0.00203	1.01×10^{-4}
Adjusted means						4	0.00204	5.10×10^{-4}
Total	25	0.022137	0.065098	0.195502		24	0.00407	1.69×10^{-4}

1. Comparison of slopes $F = 1.89 \times 10^{-4} / 7.95 \times 10^{-5} = 2.38$, not significant
   Hypothesis of parallel lines is not rejected ($0.05 < P < 0.10$)

2. Comparison of elevations $F = 5.10 \times 10^{-4} / 1.01 \times 10^{-4} = 5.04$, significant
   Hypothesis of equal elevations is rejected ($0.005 < P < 0.01$)

Appendix 8. Covariance analysis of the non-linear regression of prawn mean carapace length (y) on time (log x) for benthic caged prawns at Henderson Inlet (under salmon pens or control) and net pen reared prawns at Clam Bay (fed clam processing wastes or unsupplemented) compared to a reported natural population (Butler, 1964)

Source	dF	Sum x^2	Sum xy	Sum y^2	slope	Deviations from regression		
						dF	Sum of squares	Mean square
Clam Bay								
Unsupplemented	5	0.366	0.928	2.393	2.5377	4	0.0417	0.010
Clam fed	5	0.365	1.945	10.860	5.3286	4	0.4953	0.124
Henderson Inlet								
Control	4	0.349	1.771	9.028	5.0677	3	0.0536	0.018
Under salmon pen	4	0.349	1.971	11.440	5.6396	3	0.3257	0.108
Natural population	3	4.261	11.472	32.667	2.7555	2	0.3126	0.156
Within						16	1.2291	0.077
Regression coefficient						4	5.9538	1.488
Common	21	5.692	18.357	66.389	3.2253	20	7.1829	0.359
Adjusted means						4	4.3900	1.097
Total	25	6.467	20.592	77.145		24	11.5728	0.482

1. Comparison of slopes F = 1.488/0.077 = 19.4, significant
   Hypothesis of parallel lines is rejected (0.0005 < P)

2. Comparison of elevations F = 1.097/0.359 = 3.06
   Hypothesis of equal elevations is rejected (0.025 < P < 0.05)

Appendix 9. Covariance analysis of the non-linear regression of prawn mean weight (y) on time (log x) for the benthic caged prawns at Henderson Inlet (under salmon pens or control) and net pen reared prawns at Clam Bay (fed clam processing wastes or unsupplemented) compared to a reported natural population (Butler, 1964)

Source	dF	Sum x^2	Sum xy	Sum y^2	slope	Deviations from regression		
						dF	Sum of squares	Mean square
Clam Bay								
Unsupplemented	5	0.363	0.897	2.317	2.449	4	0.1195	0.030
Clam fed	5	0.365	2.872	23.502	7.869	4	0.8975	0.224
Henderson Inlet								
Control	4	0.349	2.272	14.981	6.501	3	0.2122	0.071
Under salmon pen	4	0.349	2.944	25.062	8.424	3	0.2654	0.088
Natural population	3	4.261	14.157	48.127	3.322	2	1.0942	0.547
Within						16	2.588	0.162
Regression coefficient						4	17.3027	4.326
Common	21	5.692	23.142	113.989	4.066	20	19.891	0.994
Adjusted means						4	17.647	4.411
Total	25	6.466	25.141	135.287		24	37.538	1.564

1. Comparison of slopes $F = 4.326/0.162 = 26.70$, significant
   Hypothesis of parallel lines is rejected ($0.0005 < P$)

2. Comparison of elevations $F = 4.411/0.994 = 4.44$, significant
   Hypothesis of equal elevations is rejected ($0.005 < P < 0.01$)

PART IV

A STUDY OF OYSTER AND MUSSEL RAFT CULTURE AND THE ASSOCIATED FOULING ORGANISMS IN HENDERSON INLET

FINAL REPORT OF 1974 EXPERIMENTS

by

Steven M. Wilson

A STUDY OF OYSTER AND MUSSEL RAFT CULTURE AND THE ASSOCIATED FOULING ORGANISMS IN HENDERSON INLET

FINAL REPORT - 1974

Steven M. Wilson

INTRODUCTION

Oyster Culture

Oysters or mussels have been harvested from most of the inhabited seacoasts of the world. In areas where the molluscs have commercial value, techniques have been developed to gather these shellfish at the larval stage and cultivate them in such a way as to increase the yield and facilitate harvesting.

The earliest means of collecting oyster seed was by simply spreading oyster shells, or cultch, throughout the intertidal areas. The larvae, or seed, are selective for the cultch in their attachment and when localized, avail themselves to management. Such bottom culture has the disadvantage of being subject to siltation and predation by various starfish, drills, crabs, and worms. The growth process is also interrupted periodically as the shellfish are uncovered by the receding tide.

Stick culture of oysters has evolved as a means of lessening the predation of bottom organisms. This method utilizes 18- to 20-inch stakes approx 1 inch in diam, with a nail driven partially into one end. The opposite end is driven into the ground and a seeded oyster cultch shell with a punched hole is placed on the nail. While reducing the mortalities from predation and siltation, the shellfish are nevertheless affected by

interrupted feeding due to tidal action.

To further provide for improved growth and survival, oysters in Europe and Japan are grown while suspended from rafts. Raft culture in Japan became established as early as 1923, and is one of the primary means of production today. The oysters are suspended on wires or ropes, known as strings, to a depth of approx 3 m, each string holding 15 to 20 single cultch shells which have been threaded by the rope or wire, and separated by hollow bamboo spacers. One raft can hold as many as 1,000 strings. (Quayle, 1969).

The advantages of raft culture are clear--continuous immersion and freedom from bottom-dwelling pests and siltation. At the same time, however, the lack of exposure at low tide may contribute to fouling problems. Even so, it has been shown that raft culture of oysters is altogether beneficial for commercial use. The time needed to attain market size is reduced from 3 years in bottom culture to 2 years. The condition index for raft-grown oysters is also higher than oysters from the bottom. An additional benefit of raft culture is that oysters may be raised in areas previously unsatisfactory due to unsuitable bottom conditions, such as found in the steep-sided inlets of Norway or British Columbia.

Mussel Culture

The culture of mussels is nearly as old as that of oysters, extending back to the 12th century in France. The use of stakes driven into intertidal areas to provide a suitable substrate for mussel larvae is

known as the "bouchot" system. As in stick culture of oysters, siltation and predation are lessened, and the crop is localized to provide easy access for cultivation and harvesting (Field, 1921).

In the 1940's, mussel growers in Spain began using rafting techniques to utilize the Galician Rias, which have steep, rocky shorelines. The rafts are capable of suspending hundreds of ropes upon which the mussel larvae attach and grow. On these ropes, extending to a depth in excess of 5 m, the mussels grow fast enough and in such densities as to warrant periodic thinning. The culled mussels are wrapped in cotton mesh which is, in turn, wrapped around bare ropes and submerged. By the time the mesh has rotted, the mussels have become attached to the ropes.

Raft culture of mussels in Europe has a high productivity with little cost. Molluscs, of course, are filter feeders with a high conversion index (Andreu, 1968). Unfortunately, the demand for mussels in North America is practically nonexistent, and mussel culture is consequently undeveloped. Because such a rich food source will undoubtedly be exploited in the future, the basis for this exploitation must be established now.

STUDY OBJECTIVES

This study project is concerned with raft culture of the Pacific oyster (Crassostrea gigas) and the common blue mussel (Mytilus edulis), two lamellibranchs of the molluscan phylum.

The objectives of this investigation are fivefold: 1) to obtain growth

curves and condition indices for C. gigas and M. edulis grown in varying proximities to the Weyerhaeuser salmon pen-rearing project to determine if the nutrient load being released within the pen-rearing area has a positive or negative effect upon shellfish growth; 2) to utilize the growth curves and condition indices to evaluate the growth potential of Henderson Inlet as compared to various other northern and southern Puget Sound oyster-growing areas; 3) to identify and quantify the fouling organisms found upon the oyster and mussel cultures; 4) to determine the relationships between fouling, growth rate and mortalities; and 5) to contribute to local ongoing studies of mussel culture, i.e., to determine periods of intensive larval attachment, the accompanying environmental conditions, and upon which substrates and at what depths attachment occurs in the greatest abundance.

The location of this study is at the Weyerhaeuser log dump on Henderson Inlet in southern Puget Sound near Woodard Bay. The salmon rearing pens have been positioned inside an outer slip of this log dump. Five stations have been established around the perimeter of the slip for monitoring the growth of both species of bivalves. In addition, three stations are located outside the pen-rearing area to function as control sites, (Figs. 1 through 5).

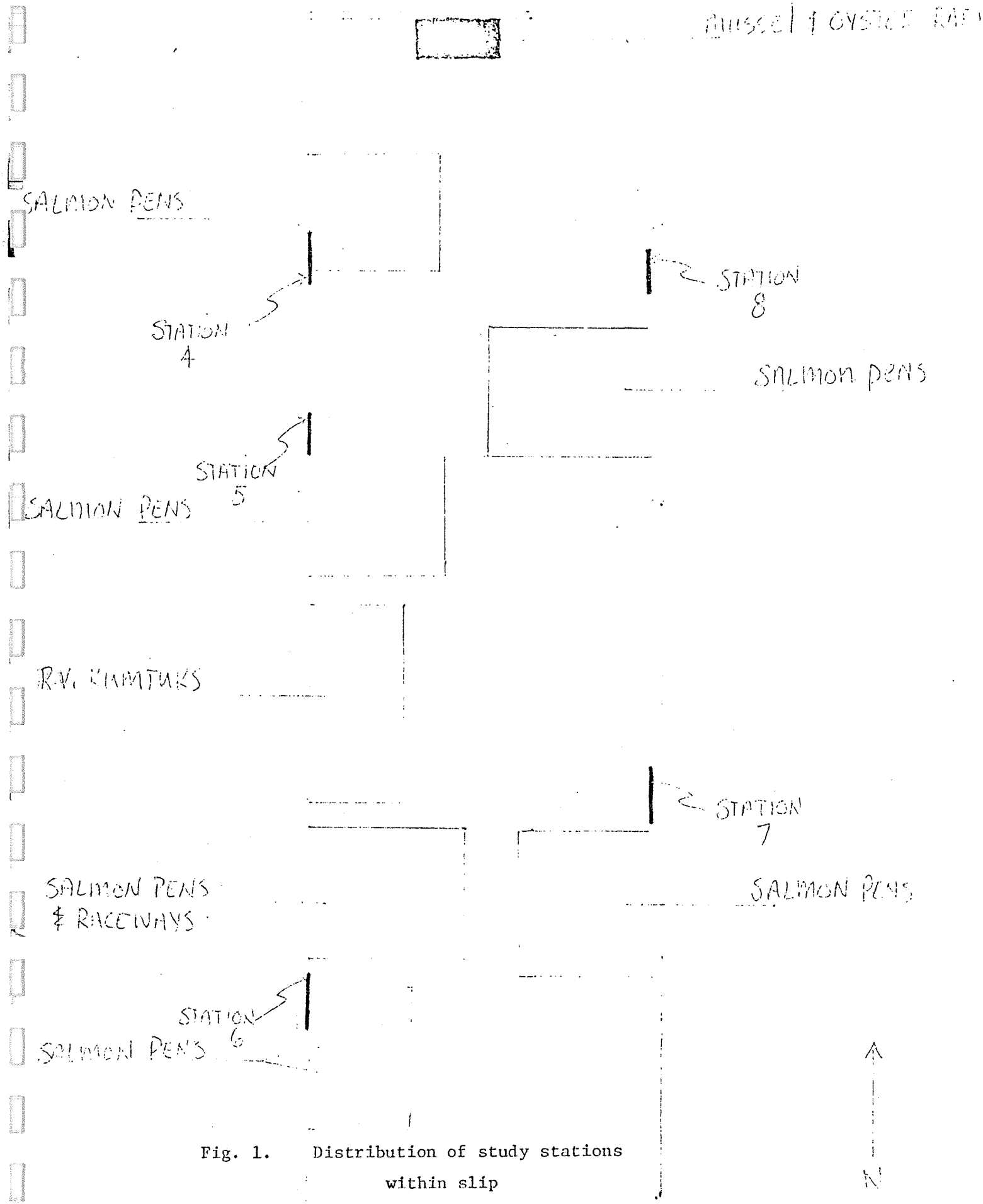

Fig. 1. Distribution of study stations within slip

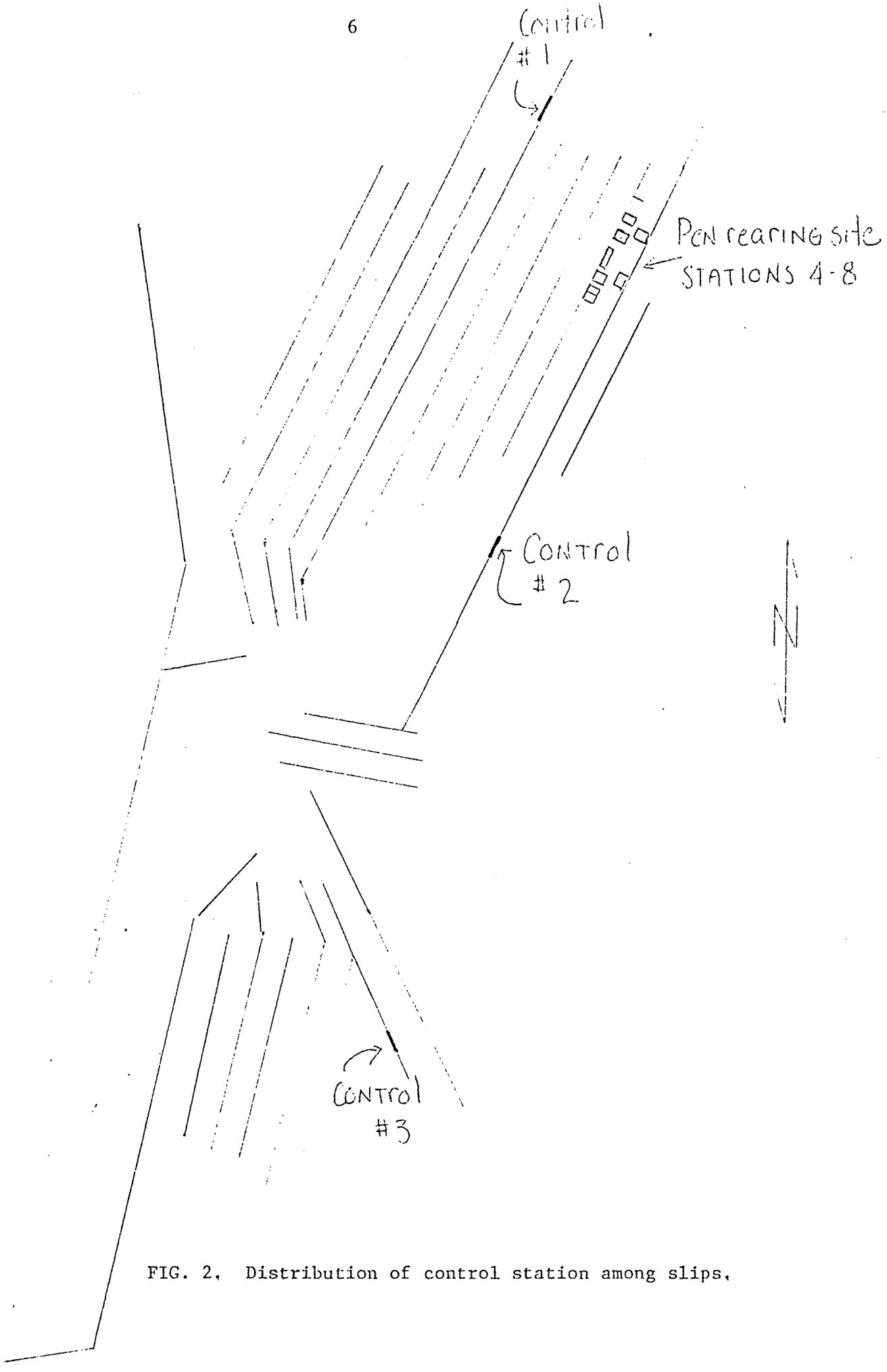

FIG. 2. Distribution of control station among slips.

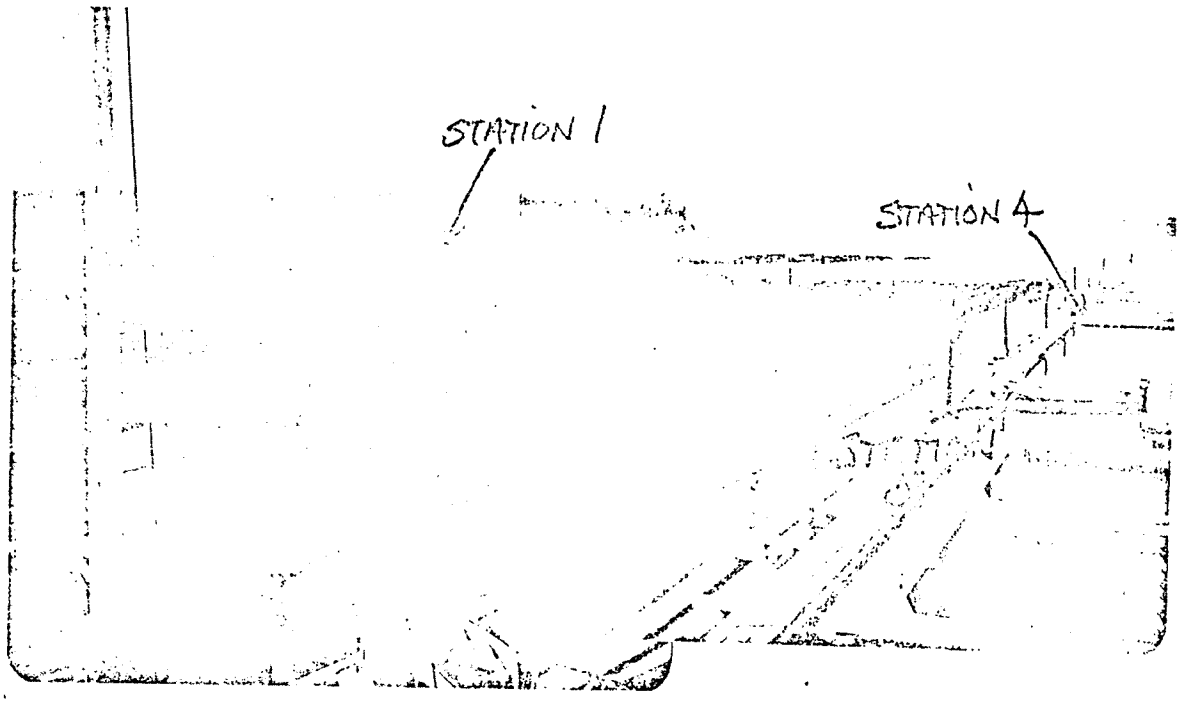

FIG. 3. Stations 1, 4, and 5, viewed north

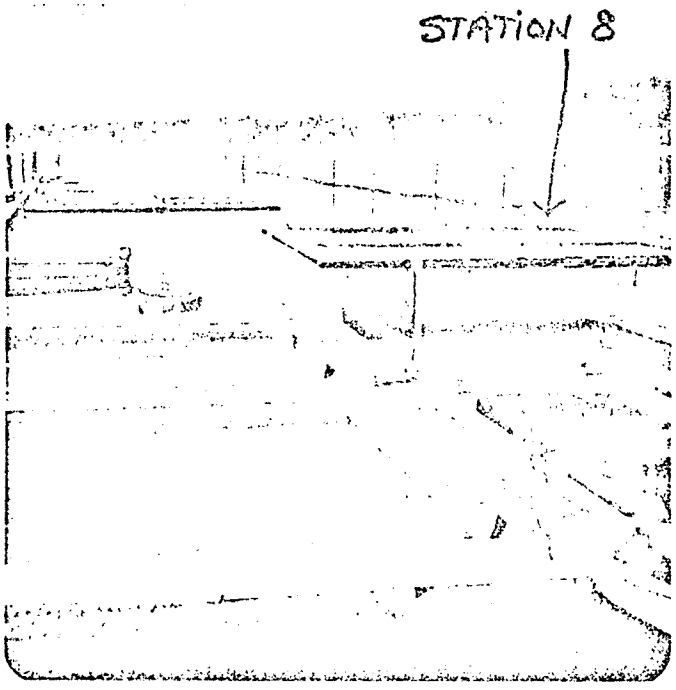

FIG. 4. Station 8, viewed north

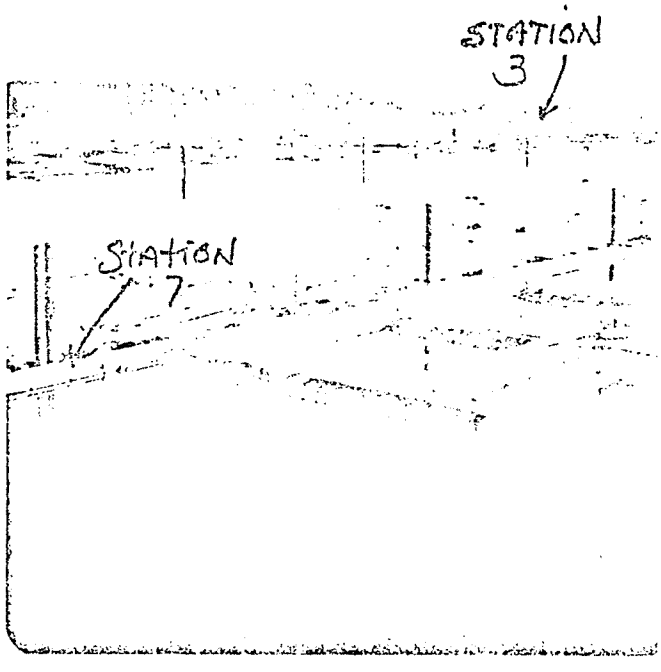

FIG. 5. Stations 3 and 7, viewed south

METHODS

Oyster Culture

On June 1, 1974, twelve rens (wire loops strung with approx 100 seeded cultch shells) were purchased at Dabob Bay on Hood Canal and taken to Henderson Inlet. The seed, which had set in September 1973, was to be strung on hanging cultures. These hanging strings are composed of a 7-ft length of No. 10 gauge soft galvanized wire, the cultch being separated by 6-inch lengths of 3/8-inch PVC tubing (Fig. 6).

To determine the variation in number of seed/cultch between rens, a group of 25 cultch shells was sampled from each ren. Each of the sampled cultch shells was examined for seed until identical counts were obtained on two successive examinations. The mean number of seed/cultch for individual rens ranged from 1.88 seed/cultch to 4.68 seed/cultch. This indicated a need for randomness in the selection of cultch shells during the construction of the strings. Due to the total number of cultch being worked with (approx 1200) this random selection from the total group was not feasible. Instead, each string was constructed with one cultch from each ren, and these cultch were then arranged on the string in a random order. It should be noted that a "commercial" set has a minimum of 10 to 12 seed/cultch; however, for the purpose of the growth studies encompassed by this project, three to four seed/cultch is adequate.

After the construction of the strings, they were distributed throughout the five inside stations, and three outside stations, with nine strings/site.

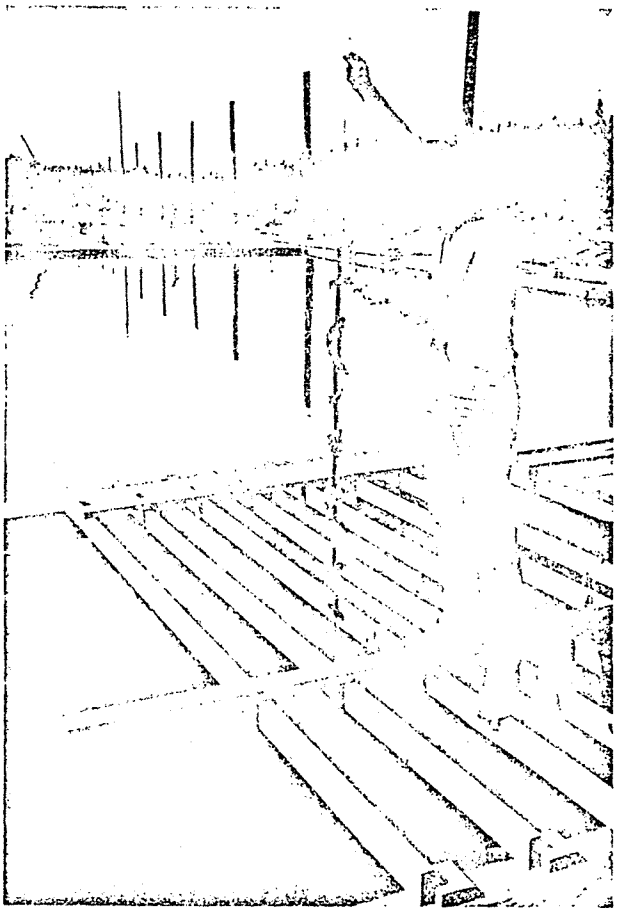

FIG. 6. Construction of hanging oyster string. Note cultch shells divided by spacers.

At each station, the nine strings were attached to stationary boom sticks.

To obtain base measurements for growth curves in the oyster culture study, one string from each station was brought into the wet lab and each cultch was thoroughly scrubbed with a soft bristle toothbrush to uncover every seed on the cultch. Using a set of carpenter's dividers, length and width measurements to the nearest mm were taken for each oyster. To establish a standardized procedure, length was designated as the distance from the umbo to the farthest reach of the shell. Width was then the greatest distance obtained on the perpendicular to the length measurement. To identify each oyster and to be able to return to the same oyster for consecutive monthly measurements, the interior of each cultch was examined first, and sampling began with the closest oyster to the cultch's umbo. The next closest seed would be the next recorded, and so on. If two were equidistant from the umbo, the oyster on the right would be sampled first. This same procedure was also used on the exterior of the cultch. Every 4 weeks, the strings are sampled for growth and a percentage of increase over the previous month is calculated. An additional indication of growth and health will be described in the discussion of mussels.

Mussel Culture

The mussel culture study is being conducted with larvae obtained from within the study site. In cooperation with local ongoing studies, three types of rope--sinclove, polypropylene (both synthetic), and manila--have been submerged at various depths to collect the larval set. The ropes are suspended horizontally on two wooden frames designed to hold 10-ft lengths of rope at specific depths. One frame holds 12 ropes,

two of each type at 0 ft and another set of six ropes at 1-ft in depth. The second frame holds 18 ropes, also suspended horizontally, at depths ranging from 0 ft to 5 ft (Fig. 7). In this situation, three ropes (one of each substrate) are held at each level. The purpose of horizontal suspension is to let the larvae set equally along the length of the rope. The inability of the mussels to migrate to various depths will give an indication of larval abundance at each depth. After 4 weeks on the frames the ropes are removed, tagged for future identification, and distributed to the eight study stations.

Consecutive growth measurements for mussels are significantly more difficult to obtain due to the fact that M. edulis can migrate, whereas oysters remain firmly attached to their substrates. The mussels also grow in the rope creases, further hindering efforts to record early growth data. Therefore, the procedures used to show growth and quality will be removal and sacrifice of some of the larger mussels. In addition to length and width measurements. these mussels will be examined for a condition index after reaching 2.0 cm in length. This index gives a value for the percentage of the shell cavity which the body parts occupy (Baird, 1958). Two variations of condition index are available. The more exacting method calls for the meat to be dried to a constant weight at 100 C before being used to compute percentage of volume. This method uses the formula:

$$\frac{\text{weight of dried meat (g)}}{\text{shell cavity (ml)}} \times 1000 \text{ (Medcof and Needler, 1941)}$$

Variations of this technique have been used by other researchers. Baird

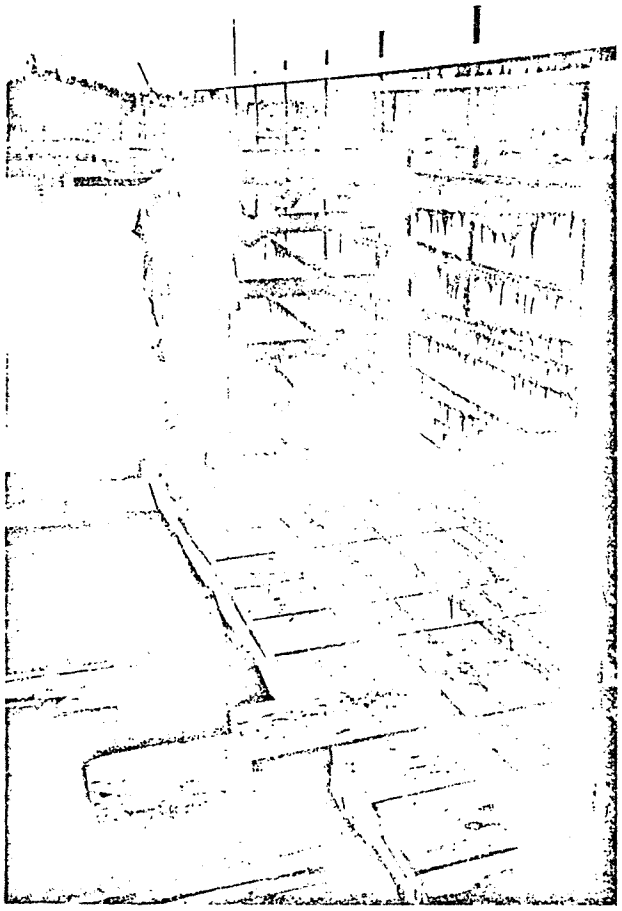

FIG. 7. Frame utilized in suspending three types of rope from depths of 0 ft to 5 ft to catch mussel larvae.

(1958) expressed the belief that the inherent variability found in samples of mussels and oysters demands the use of large samples. To facilitate the use of larger N values (50 or greater), Baird assessed the condition index by dividing the wet volume of the meat by the volume of the shell cavity and multiplying by 100.

$$\frac{\text{meat volume}}{\text{shell cavity volume}} \times 100 = \text{condition}$$

This method will be used to assess the condition index for mussels and also for oysters in this study, the mussel being sampled bimonthly, while the oysters are to be sampled every 6 months.

Fouling Studies

In each culture study, the fouling organisms are being collected, identified, and cataloged as predators, competitors, or harmless. The organisms are collected from the oysters during the cleaning in preparation for the condition indices. In the periods between these cleanings, the succession of algal, hydroid, and diatom communities is being studied. The same investigations are being conducted to identify fouling organisms inhabiting the ropes.

RESULTS

As a consequence of beginning this study on June 2, 1974, much of the potential mussel larvae set and growing season for both mussels and oysters were lost. However, in the four month period that the study was conducted, three sets of oyster growth data have been taken and visual observations give definite indications of mussel setting and growth.

Oyster Culture

Four months is an insufficient period of time to make a comparative analysis of growth among sites and, as summarized in Table 1, the growth in all areas is quite varied. The lack of measurements at Station 3 on July 9 is due to loss of the entire study group, possibly due to vandalism. Initial measurements were re-taken on a new set of cultch on August 13 and the study site was relocated to a less exposed area. The high mortality rates at Station 1 (August 13) and Station 7 (September 11) were due to predation by starfish. It is not known how the predators attached at Station 1, but those at Station 7 were picked up when the string was torn from the boom stick and fell to the bottom for a period of 3 days.

To obtain a feeling for early growth of the seed, compared to other areas of Puget Sound, opinions concerning the size and condition of the raft-grown oysters were requested from Mr. Guy Bever, Western Oyster Co. and Mr. Donald Nelson, an independent oyster grower. Mr. Bever felt that the oysters were somewhat smaller than Dabob Bay seed of the same age which had been transported to Puget Sound immediately after setting, such as those which he is currently growing. However, he felt that after a short acclimatization period, the shellfish would exhibit rapid growth. Both he and Mr. Nelson felt the oysters displayed a high degree of fatness, i.e., a good condition index.

Table 1. Oyster growth data, Henderson Inlet, June 1 to September 30, 1974

	Adjusted average* length and width (mm)	Monthly growth increase (%)	Percentage mortality (cum)
Station 1			
July 9	20.5	-	-
August 13	25.9	94.4	11.3
September 11	32.8	75.1	14.5
Station 2			
July 9	21.5	-	-
August 13	25.1	47.7	1.5
September 11	31.1	57.3	4.5
Station 3			
July 9	-	-	-
August 13	22.4	-	-
September 11	32.1	163.0	12.3
Station 4			
July 9	21.1	-	-
August 13	28.5	105.0	1.8
September 11	35.7	60.1	1.8
Station 5			
July 9	19.9	-	-
August 13	25.7	92.2	1.7
September 11	31.3	53.6	1.7
Station 6			
July 9	18.7	-	-
August 13	23.3	84.9	0.0
September 11	28.7	71.5	0.0
Station 7			
July 9	21.3	-	-
August 13	26.7	73.7	2.3
September 11	32.0	45.9	59.1
Station 8			
July 9	22.5	-	-
August 13	27.2	50.3	0.0
September 11	27.5	32.9	7.5

* The monthly increases in growth are determined by multiplying length x width. To gain the average length and width measurement, the mean of these length x width values is found, and its square root is used. Obviously, all oysters do not have the same linear value for both length and width

Mussel Culture

A mussel set occurred during the June-July period and the larger mussels are now nearly 2.0 cm in length.* If a sufficient sample of mussels greater than 2.0 cm in length can be obtained, condition indices will be recorded in the month of October. The second and third groups of ropes (July to August, and August to September) appear to have had little, if any, set. It is not possible to accurately enumerate the set until the larvae are more fully developed (> 5 mm).

Preliminary observations indicated that during the June to July set, attachment occurred throughout the water column with equal abundance. Observations since that time have determined that this may not be correct. There is definitely a greater distribution of mussels on those ropes which were suspended at the surface than on those ropes suspended below. Moreover, when those ropes which were suspended horizontally are hung vertically, the greatest growth is observed in the upper 15 cm of the water column. Another indication of unequal larval distribution is found in the fouling of cultch shells. Those nearest the surface have the densest mussel attachment, while those cultch progressively deeper have fewer mussels.

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* September 1974

DISCUSSION

Three areas of study should be included in the next growing season:

1. A study of the relationship of fouling to growth and survival in oysters will be accomplished by constructing new strings with 1974 seed and periodically immersing the experimental groups in a brine solution to control the fouling growth. A control group will be left untouched. After a 6-month period, the condition indices and mortality rates of each group will be computed and compared.

2. The horizontally suspended ropes, upon which mussel larvae attach, will be left on the frames for 3-month periods to avoid losing attached larvae during transfer from frame to stations. A strong possibility exists that many of the attached larvae from the June 1974 to July 1974 set were lost in transit.

3. Two thermographs will be attached adjacent to the ropes at depths of 15 cm and 30 cm to determine the temperature regime of the upper water column.

An additional modification may be needed in the study design of this project, that being relocation of the study sites. Two problems have occurred since the inception of this study. The first is acceleration of the fouling through contact with the boomsticks, and the second is loss of ropes and strings due to vandalism, and activities associated with rafting of log booms.

Fouling

The preliminary fouling organism occurring on both the mussel ropes and oyster strings is a hydrozoan, possibly Obelia longissima. Other prevalent organisms found primarily on the oyster cultures include ascidians, both solitary (Corella willmeriana) and compound (Distaplia occidentalis); Melosira, an intertidal diatom; bryozoans such as Bugula, an arborescent form, and Membranipora membranacea and Schizoporella bicornis which are encrusting bryozoans; amphipods, primarily Caprella equilibia; and various polychaetes as yet unidentified. On the mussel ropes, the dominant organisms other than Obelia are compound ascidians and encrusting bryozoans. It remains to be seen whether the communities will remain year around, or whether they will be replaced by a climax growth such as mussels or barnacles.

CONCLUSION

This study was terminated in the fall of 1974 and it was not possible to determine whether the growth rates of C. gigas and M. edulis are affected by the nutrients released in the pen-rearing area. Many factors are as yet unknown, such as what effect the weathering of ropes has upon mussel attachment, and how fouling affects the development of shellfish.

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CONCLUSION

The Henderson Inlet area appears to be suitable for the mariculture of coho salmon and proper immunization by either intraperitoneal or vacuum infiltration methods furnishes adequate protection against *Vibrio anguillarum*.* Results in 1974 with chinook salmon were not as promising as in 1973. Although immunization provided protection, the growth rates in 1974 were not favorable. Whether this was a result of meteorological and hydrological conditions is not clear (1974 was a warmer year than 1973). The rearing methods were not the same and chinook salmon may require more intensive, and thus more costly, care.

Although there were no significant effects upon the water quality, there were measurable effects upon the benthos beneath the salmon-rearing pens. Once the pens were removed, the recovery was rapid, so a management strategy should include a rotation plan for the pens as well as using locations with the greatest flushing rates compatible with maintenance of the pens.

Polyculture of spot prawns is a possibility in salmon pens, particularly when the prawns have access to deeper waters. The surface waters at Henderson Inlet are limiting for prawns because protozoan fouling and dense blooms of plankton during some years are not compatible with the culture of prawns. Northern Puget Sound and Hood Canal are apparently more suitable for the rearing of prawns.

The experiments with oysters and mussels were incomplete and were terminated before any judgment could be made. The area can and does support oysters and mussels.

Ernest O. Salo

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*In 1974, only the intraperitoneal method was used; in 1975 both methods were used.