THE USE OF HEATED SEAWATER FOR FARMING OYSTERS AND SALMON

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SUMMARY

Oyster Growth Studies

(1) The objective of these studies was to determine the biological feasibility of using the heated effluent from coastal nuclear power plants for culturing the Pacific oyster, Crassostrea gigas.

(2) Three general types of experiments were carried out, these included: (1) oyster growth experiments in which the growth rates of juvenile oysters and spat were determined under various combinations of water flow rate and temperature, (2) seasonal growth studies intended to show how oyster growth varies with season, and (3) closed system studies in which oysters were held in a large recirculating seawater system and provided with cultured algae as food.

(3) Oyster growth studies were conducted both at Port Orford, Oregon, an open coast location, and in Newport, Oregon, an estuarine location. The studies showed no consistant growth or survival advantage to either location indicating that culturing oysters at an open coastal site is biologically feasible.

(4) The relationship between water flow rate and oyster growth is highly variable. This variability is caused primarily by fluctuations in the concentration of food in the water. Consequently, no generally applicable water flow requirement for oyster growth can be given. In our experiments with juvenile oysters good growth was obtained with flows of from 20 to 40 ml/min/oyster.

(5) In general, improved growth was obtained using temperatures up to 15°C. For an open coastal location in Oregon this is about 4-6°C above ambient.

(6) With two exceptions we found little or no growth advantage to temperatures exceeding 15°C. The exceptions are; (1) shell growth in spat, and (2) meat growth in juveniles during periods of the year when food is extremely plentiful.

(7) Our data show that if increased temperatures (exceeding 15°C) are accompanied by decreased food availability, due to overcrowding, inadequate water flow rates, or seasonal changes in the food content of the water, reduced growth and high mortality will result.

(8) Observation of seasonal fluctuations in oyster growth indicates that little or no growth occurs between October and March. In one experiment for example we found that 86% of the oysters' yearly growth occurred in a six month period from April to September.

(9) Our evidence indicates that this "no-growth" period is due primarily to a lack of food, not to reduced salinity and temperature during the winter.

(10) Preliminary experiments have been conducted using a closed system to obtain data necessary for testing supplemental feeding during the periods of low natural food. Control of mortality and provision of a qualitatively adequate diet have proven to be our most serious problems in these studies.

Salmon Growth Studies

(1) The objective of these studies was to determine the biological feasibility of using the heated effluent from coastal nuclear power ants for culturing chum salmon, Oncorhynchus keta, and, to a lesser extent, pink salmon O. gorbuscha.

(2) Three general types of experiments have been completed. These are; (1) temperature vs. growth experiments using chum and pink salmon carried out at the Port Orford laboratory, (2) temperature x ration factorial experiments with chum salmon only carried out in Newport, and (3) disease control studies.

(3) Limited studies with pink salmon indicate that they may be faster growing than chum salmon, and that the pinks may be able to tolerate higher temperatures than chums.

(4) In general, our best growth was obtained at about 14°C for chums and for larger pinks. Very small pink salmon (less than 50 g wet weight) grew best at 18°C. Therefore, since ambient seawater temperatures are about 10°C, some growth advantage could be realized by heating the water.

(5) Disease proved to be a serious problem in all experiments. Bacterial Kidney Disease, caused by Corynebacterium sp., was prevalent in the Port Orford experiments. Vibriosis, caused by the bacterium Vibrio anguillarum, caused mortalities in the Newport experiments.

(6) Disease problems were invariably aggravated by any stress condition, including improper feeding and high temperatures.

(7) Efforts to control vibriosis by vaccination met with only limited success. Since the only reliable way to control vibriosis currently is to avoid stress conditions, it is extremely unlikely that chum salmon could be commercially cultured at temperatures that consistantly exceed 14°C.

INTRODUCTION

The following is a report of the current status of a study of the feasibility of utilizing the heated effluent from coastal nuclear power plants for the culture of salmon and oysters. The study was carried out by Oregon State University, Department of Fisheries and Wildlife with assistance from a number of other departments. Portland General Electric, Pacific Power and Light and the Eugene Water and Electric Board jointly funded the study. Additional support was provided by the National Oceanographic and Atmospheric Administration through its Sea Grant program. Work was begun in the spring of 1971. All of the work described in this report was completed prior to January 1, 1976.

This report contains summaries of all research concerning the use of heated seawater for culturing salmon and oysters carried out during the last four years by O.S.U.'s Department of Fisheries and Wildlife and receiving at least partial support from Sea Grant and the three utilities given above. Some of the work has been or will be discussed in considerably more detail in a number of graduate student theses (listed below). The significant features of all previous studies are summarized in this report. However, our more recent findings and data that does not yet appear in either a thesis or another publication are discussed with greater detail. Some of our conclusions, based on new studies, may differ to a certain extent from statements made earlier based on preliminary studies.

Data contained in this report are intended for the use of the granting agencies only. Since some of the data constitute a portion of four

graduate theses (those of Gerald Rowan, Bernard Kepshire, Hisashi Ishyama, and Robert Malouf), publication elsewhere must have the prior approval of Oregon State University's Department of Fisheries and Wildlife.

This type of work, funded primarily by Sea Grant, is still in progress at O.S.U. Significant advances in our understanding of the growth and disease phenomena under study, as well as in our culture methodology, as a result of these studies are certainly possible.

OYSTER GROWTH STUDIES

Introduction

Research dealing with oyster growth at elevated temperatures was begun at the Marine Science Center in Newport in 1970 and at the Marine Research Laboratory in Port Orford in 1971. In general terms this work was intended to assess the biological and economic feasibility of utilizing the heated saline effluent from coastal nuclear power plants for commercial oyster culture. To that end research has been conducted to define the relationship between water flow rate and oyster growth at various temperatures. These relationships were intended, then, to provide a means of estimating the capacity of unenriched seawater at various temperatures to support the growth of oysters.

Concurrent with the temperature x flow rate studies, experiments were conducted both at Port Orford and Newport to determine the magnitude of seasonal fluctuations in oyster growth at a given water flow rate.

A third type of experiment using a closed, recirculating seawater system has also been initiated. These closed system experiments, which are in fact only in their preliminary stages at this time, will provide us with data concerning the food consumption, oxygen consumption, assimilation efficiency and growth of oysters held at a number of different temperatures and provided with carefully controlled quantities of food in the form of cultured algae. These experiments will also provide us with data necessary to evaluate the feasibility of using cultured algae or some other food to supplement natural food during known periods of low food availability.

Preliminary Studies

Since it seemed unlikely that any nuclear powered generating plant in Oregon would be located on an estuary, preliminary work conducted for the most part at the Port Orford laboratory was designed to determine if juvenile oysters would grow or even survive in the full strength seawater of the open coast. The work was also intended to determine what effect temperature might have on growth and survival under conditions in which water flow was not limiting.

These initial experiments provided evidence that juvenile oysters survive well in high salinity water and that their growth can be enhanced at elevated temperatures.

This work also provided our first indications that the growth of oysters at any temperature varies considerably with season. Details of our finding with regard to season are given in a following section of this report.

Growth Experiments

A series of four experiments using cultchless juvenile oysters and one using spat on cultch were carried out in Port Orford and Newport between January of 1973 and July of 1974. In the descriptions and discussions that follow, the experiments are designated as follows:

Experiment I - Port Orford . . .Jan. 16, 1973 - March 12, 1973

Experiment I - Newport . . . . .Jan. 23, 1973 - March 18, 1973

Experiment II - Port Orford . . .April 7, 1973 - June 22, 1973

Experiment II - Newport . . . . .March 30, 1973 - June 22, 1973

Spat Experiment I - Port Orford . . May 27, 1973 - June 24, 1973

Spat Experiment I - Newport . . . . May 25, 1973 - June 22, 1973

Experiment III - Newport . . . . . Oct. 11, 1973 - Dec. 15, 1973

Experiment IV - Newport . . . . . .May 15, 1974 - July 17, 1974

These experiments will be described in chronological order to provide some understanding of the rationale behind successive design changes.

All of these experiments, except those designated as "spat" experiments, were conducted with single "cultchless" oysters of a relatively uniform initial size. In the spat experiments, smaller oysters (spat) attached to shell cultch were used.

Experiment I - Port Orford Jan. 16, 1973-March 12, 1973

Objective - This experiment had two objectives. These were: 1) to investigate the relationship between water flow rate, temperature, and oyster growth; and 2) to provide growth data for comparison with data from oysters grown concurrently in Newport.

Design - Basically, the experiment was a 4 x 4 factorial design (four temperatures and four water flow rates in all possible combinations). The temperatures used were 10°, 15°, 18°, 21°C. Water flow requirements for this first experiment were estimated from a broad range of values that appear in the literature (75 to 175 ml/min/50 oysters for oysters of the

size used). We used flows of 50, 100, 200, and 400 ml/min/50 oysters to cover the range of values reported in other studies.

Shell growth, as shell length only, was determined on the basis of biweekly measurements of 35 randomly-selected oysters from each of the 16 treatments. Meat growth, as a change in wet meat weight, was determined by weighing the meats from 50 oysters randomly selected from the same large group and at the same time as the experimental animals. Then, at the end of the experimental period, 35 oysters from each treatment were shucked and weighed. Growth was expressed as the difference between the initial mean weight and the final mean weight for each treatment. This method for estimating changes in meat weight was used with little modification in all of the experiments described in this report.

Results - Shell growth was found to be minimal in all treatments; there was essentially no shell growth during the experiment. The changes in meat weight, both positive and negative, are shown in Table 1. As with shell length, meat growth (gain or loss) was very slight during the 40 day experiment. In fact the most important feature of this experiment is the failure of any temperature x flow rate combination to produce significant growth.

Mortalities during the experiment were quite high (Table 2), and were somewhat higher at the higher temperatures.

We can speculate that under conditions that are nutritionally inadequate, as our growth data show these to be, factors such as high temperature, that contribute additional stress on the animals increase their rate of mortality.

Table 1. Port Orford Experiment I. Jan. 16-March 12, 1973. Change in wet meat weight (initial wet meat weight = 0.18 g).

Temp. °C	Flow (ml/min/50 oysters)				
	50	100	200	400	$\bar{x}$
10	+0.04	-0.01	+0.05	+0.06	+0.04
15	-0.01	-0.02	0.00	+0.02	0.00
18	0.00	-0.01	+0.02	-0.01	0.00
21	+0.01	0.00	+0.02	-0.01	+0.01
$\bar{x}$	+0.01	-0.01	+0.02	+0.04	

Table 2. Port Orford Experiment I. Jan. 16-March 12, 1973. Total percent mortality over 40-day experimental period.

Temp. °C	Flow (ml/min/50 oysters)				
	50	100	200	400	$\bar{x}$
10	26	26	30	28	28
15	36	22	34	38	33
18	28	40	40	46	39
21	32	34	54	58	45
$\bar{x}$	31	31	40	43	

Experiment I - Newport Jan. 23, 1973-March 18, 1973

Objective - To provide a growth and survival comparison with open-coast waters (Port Orford).

Design - Basically, what we attempted to do in this experiment was to duplicate in Newport a portion of the experimental array that we had in Port Orford and to run an experiment concurrently with the Port Orford experiment.

The oysters used in the experiment were randomly drawn from the same "pooled" group as the Port Orford oysters. The oysters were placed in each of four stacks of five trays (modified Heath incubators). Each stack received water of a different temperature (10°, 15°, 18°, 21°C), but at the same flow rate. Since the water received by each stack of trays flowed down from one tray to another, only the upper tray in a stack was considered comparable to the Port Orford experiment. Therefore, only growth measurements made on the upper tray in the stack are reported here.

The water flow rate used for each temperature was 1,000 ml/min. Since 125 oysters were used in the first tray the water flow per oyster was equivalent to the highest flow rate used in Port Orford (8 ml/min/oyster).

Results - Growth in this experiment was measured on the basis of increase in shell length only. The results of these measurements are given in Table 3. As in the Port Orford experiment, growth was slight and not clearly related to temperature.

Table 3. Newport Experiment I. Jan. 23-March 18, 1973. Shell growth at four temperatures. (Water flow = 8 ml/min/oyster).

Temp. °C	Initial length (mm)	Final length (mm)	Change in length (mm)	Percent increase
10	26.4	28.7	+2.3	8.7
15	24.8	27.1	+2.3	9.3
18	26.6	28.3	+1.7	6.4
21	25.8	27.7	+1.9	7.4

Table 4. Newport Experiment I. Jan. 23-March 18, 1973. Effect of temperature on mortality.

Temp. °C	Total mortality	Percent mortality
10	14	9.3
15	53	35.3
18	57	38.0
21	73	48.7

Mortality data, given in Table 4, show an increase in mortality at elevated temperatures. This pattern of mortality was, again, probably a response to the stress of low food availability combined with increased temperatures.

Experiment II - Port Orford April 7, 1973-June 22, 1973

Objective - The second pair of experiments was essentially a repeat of the first pair. The objectives and design were, with few exceptions, unchanged.

Design - As in Experiment I but with minor improvements in the apparatus to provide improved reliability. The oysters used were drawn from the same group as the first experiment. Oysters used in the first experiment were not returned to the pooled group at the termination of the experiment. So, although the source was the same, different oysters were used in the two experiments.

Results - Table 5 shows the change in mean wet weight for each of the 16 combinations of temperature and flow used in Experiment II. The results of the experiment were very similar to the first Port Orford experiment (compare Tables 1 and 5) as far as general relationships are concerned, but growth was somewhat better in the second experiment. As in the first experiment, the combination of temperature and flow that yielded the best growth and the highest percent survival (Table 6) was the lowest temperature and the highest flow 10°C x 400 ml/min/50 oysters). Meat growth showed a consistant inverse relationship with temperature and a direct relationship with flow rate.

Table 5. Port Orford Experiment II. April 7-June 22, 1973. Change in mean wet meat weight (growth) in grams. Initial wet meat weight was 0.22 g.

Temp. °C	Flow (ml/min/50 oysters)				
	50	100	200	400	$\bar{x}$
10	0.00	-0.03	+0.11	+0.24	+0.08
15	-0.05	+0.02	+0.06	+0.22	+0.06
18	0.00	0.00	+0.03	+0.07	+0.03
21	-0.05	+0.02	-0.01	+0.07	+0.01
$\bar{x}$	-0.03	+0.01	+0.05	+0.15	

Table 6. Port Orford Experiment II. April 7-June 22, 1973. Percent mortality for each of the 16 temperature x flow combinations used.

Temp. °C	Flow (ml/min/50 oysters)				
	50	100	200	400	$\bar{x}$
10	16	20	22	16	19
15	44	30	38	40	38
18	80	70	66	46	66
21	74	88	68	66	74
$\bar{x}$	54	52	49	42	

Mortalities were quite high at the two high temperatures, particularly at the lower water flows. The indications are that this extreme mortality is a stress phenomenon. Plotting growth rate against water flow (Fig. 1) shows that even our highest flow rate cannot be considered be be excess. Even at the lowest temperature there is no indication from our data that 400 ml/min/50 oysters is a sufficient volume to support maximum growth. We may surmise, then, that at higher temperatures the oysters were receiving an inadequate water flow and were further stressed by elevated temperatures. This stress combination was reflected in increased mortality and reduced growth.

It seems from these experiments that the water flow data appearing in the literature and around which the experiments were designed, grossly underestimate the water requirements of oysters in an open coastal location.

Experiment II - Newport March 30-June 22, 1973

Objective - As in Experiment I - Newport.

Design - With minor improvements, unchanged from Experiment I - Newport. Unlike Experiment I, meat weight data were taken for this experiment to provide a better comparison with Experiment II - Port Orford.

This experiment, again done in modified Heath Incubators, utilized stacks of three trays at each temperature. Each stack received 1 l/min of seawater for 125 animals/tray. The water passed through tray 1 before entering tray 2, and through tray 2 before entering tray 3. As in Experiment I, only the top tray (tray 1) is comparable, therefore, to

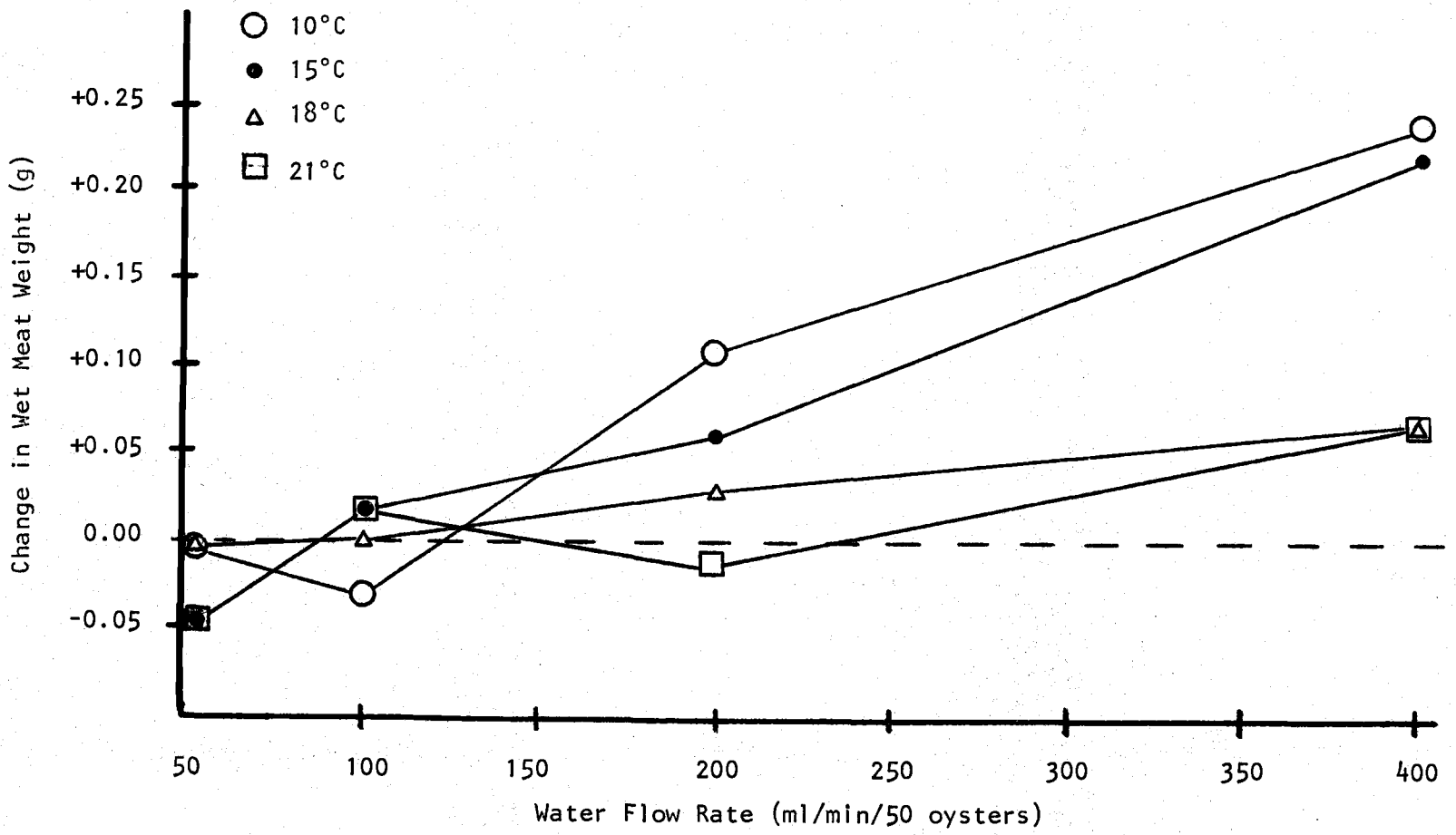

Figure 1. Relationship between water flow rate and change in wet meat weight of oysters held at four temperatures. Port Orford Experiment II, April 7-June 22, 1973. The oysters had a mean wet meat weight of 0.22 g at the start of the experiment.

conditions established in Port Orford. Results of growth and survival determination for the two lower trays at each temperature are included here to show, as we concluded, that this cascade type of culture system is probably not an efficient design for oyster culture.

Results - Growth data from this experiment (Table 7) show some important differences between our Newport and Port Orford water sources. Growth in Experiment II was considerably better than in previous experiments (compare Tables 3 and 7) and was certainly better than the growth observed in our concurrent Experiment II in Port Orford (compare Tables 5 and 8). Since other factors remained relatively unchanged (temperatures, flows, etc.) the improved growth was probably due to an increase in the food content of the Newport water during the spring months.

Notice (Table 9) that the apparent increase in natural food was also reflected by a significant reduction in mortality (compare Tables 4 and 9). Keeping in mind that the water flow in these experiments was from tray 1 to tray 2, etc. further evidence of the effects of food and temperature stress is provided in Table 9. Note, for example, the great difference in mortality between the first tray position, in which food was at least adequate for growth, and subsequent tray positions. Table 9 also shows an added stress from elevated temperatures so that the least growth and highest mortality is found at the highest temperature (21°C) and the lowest tray position.

Spat Experiment I - Port Orford May 27-June 24, 1973

Objective - To determine the influence of temperature on shell growth and survival of oyster spat held in a water flow rate considered

Table 7. Newport Experiment II. March 30-June 22, 1973. Shell growth (length in mm). Flow of 1l/min/125 oysters. Comparable with highest flow rate in Port Orford Experiments I and II (8 ml/min/oyster) and with Newport Experiment I.

Temp. °C	Initial length (mm)	Final length (mm)	Increase (mm)	Percent increase
10	26.4	32.0	5.6	21
15	26.1	32.5	6.4	25
18	25.1	31.1	6.0	24
21	25.6	30.1	4.5	18

Table 8. Newport Experiment II. March 30-June 22, 1973. Change in wet meat weights, in grams, as influenced by temperature and tray position. Water flow was from tray 1 through 3 in sequence at each temperature.

Temp. °C	Tray Position		
	1	2	3
10	+0.22	+0.08	-0.03
15	+0.32	+0.02	-0.04
18	+0.30	0.00	+0.02
21	+0.35	+0.08	-0.07

Table 9. Newport Experiment II. March 30-June 22, 1973. Percent mortality as influenced by temperature and tray position.

Temp. °C	Tray position			
	1	2	3	$\bar{x}$
10	13	30	28	24
15	14	44	42	33
18	14	50	48	37
21	14	68	86	56
$\bar{x}$	14	48	51	

to be excess for their requirements. To provide a comparison with the growth of larger, single oysters held under identical conditions at the same time, and, similarly, with spat being grown in Newport.

Design - Two hundred spat attached to flat shell pieces were selected for their relatively uniform size and even distribution on the shells. All other spat were removed from the shells. Shell pieces holding a total of 50 spat were placed in shallow trays receiving 400 ml/min at each of four temperatures, 10°, 15°, 18°, and 21°C. Weekly measurements were made of the length and width of all of the 50 spat at each temperature.

Results - Growth and mortality data for this experiment (Table 10 and Fig. 2) shows exactly the opposite relationship found with larger oysters kept at Port Orford at the same time (Table 5). The data indicate that the shell growth of spat can be enhanced considerably by elevated temperatures, and that as long as flows (food) are adequate survival is not adversely affected by the higher temperatures.

Recall that the larger oysters being held under the same conditions at the same time showed little or no shell growth and suffered high mortalities at higher temperatures. The positive effect of temperature on the smaller oyster lends support to our previous statement that the negative influence of elevated temperatures on the larger oysters was associated with an inadequate food supply, and was not a simple temperature effect.

Table 10. Port Orford Spat Experiment I. May 27-June 24, 1973. Shell growth and mortality of attached spat as influenced by temperature. Flow rate of 400 ml/min/50 spat. Values are means of 50 measurements.

Temp. °C	Initial length	Final length	Increase (mm)	Percent increase	Percent mortality
10	4.7	6.6	1.9	40	6
15	4.1	9.3	5.2	127	10
18	4.1	10.3	6.2	151	2
21	4.1	10.5	6.4	156	0

Table 11. Newport Spat Experiment I. May 25-June 22, 1973. Shell growth and mortality of attached spat as influenced by temperature. Flow rate of 400 ml/min/50 spat. Values are means of 50 measurements.

Temp. °C	Initial length	Final length	Increase (mm)	Percent increase	Percent mortality
10	3.7	5.7	2.0	54	2
15	3.7	5.9	2.2	59	5
18	3.9	6.7	2.8	71	0
21	3.7	5.8	2.1	56	2

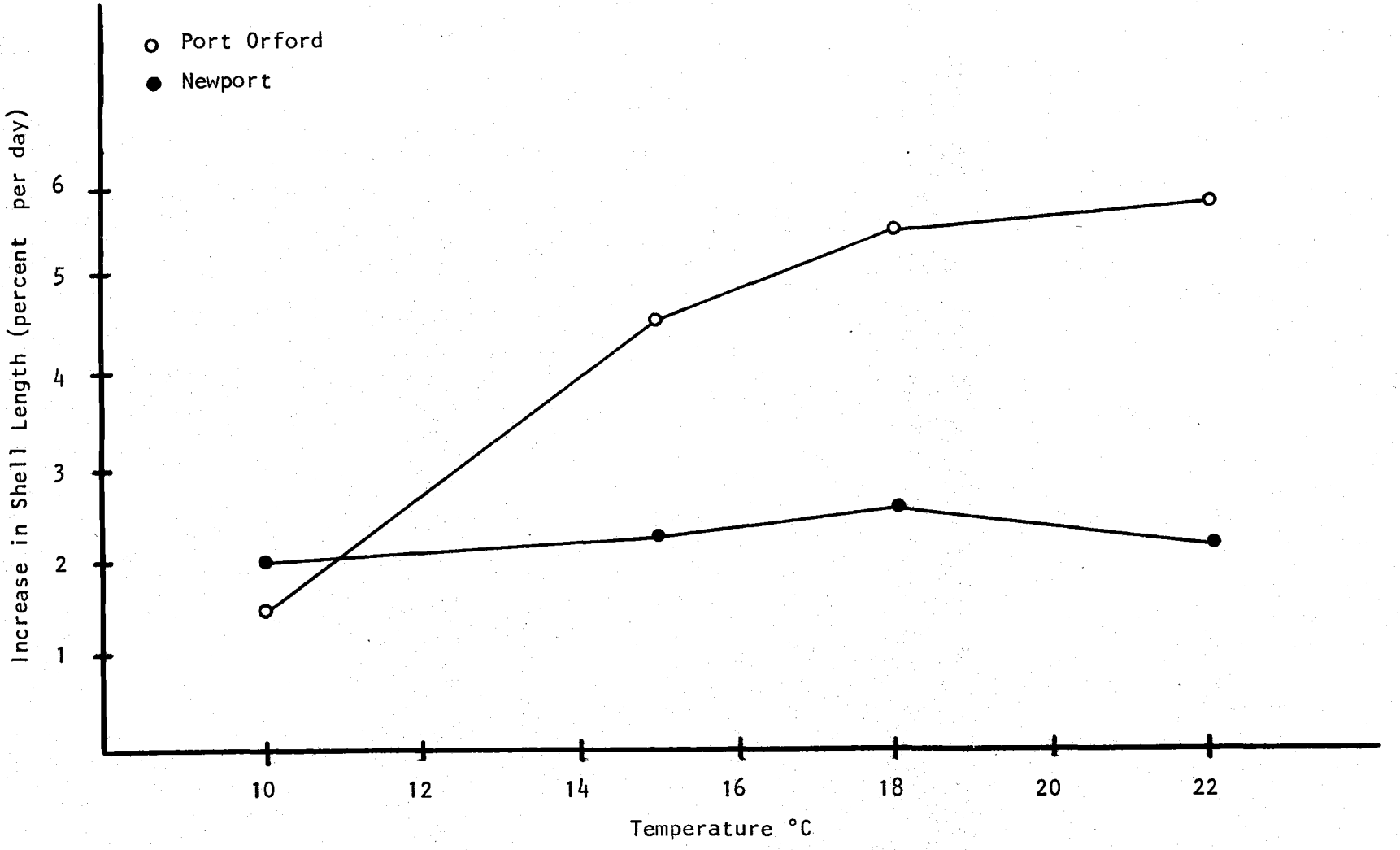

Figure 2. Relationship between temperature and shell growth of oyster spat (about 4 mm initial length) held in Port Orford and Newport. May 25-June 22, 1973.

Spat Experiment I - Newport May 22-June 24, 1973

Objective - As in Port Orford Spat Experiment I.

Design - As in Port Orford Spat Experiment I. Spat used were taken from the same stock and at the same time as the Port Orford experiment.

Results - Spat growth in this experiment (Table 11 and Fig. 2) showed the same general trend as Newport Experiment II, which was conducted concurrently with larger oysters (Table 7). Unlike the Port Orford spat experiment, the Newport experiment did not show any particular growth advantage due to elevated temperatures. On the other hand there was no evidence of reduced growth or increased mortality at higher temperatures.

Experiment III - Newport Oct. 11-Dec. 15, 1973

In an effort to refine our estimate of the temperature x water flow x oyster growth relationships that have been previously discussed, we initiated a new series of experiments in Newport in the fall of 1973. The emphasis in this series of experiments was on improving our measurement of oyster growth and on efforts to assess the food content of the water by measuring certain parameters directly.

Objective - The experiment consists of a number of parts each having its own objective and contribution to the more general purpose of the experiment. The objective of the experiment as a whole was to provide data that will permit improved definition of the water flow requirements of oysters at various temperatures.

Design - The experiment consisted basically of two separate factorial designs. The first of these was a temperature (11°C, 15°C, 20°C) x water flow (100, 200, 400, 800 ml/min/25 oysters) factorial design. Note that the water flows per oyster used in this experiment are as much as 4 times greater than those previously used for oysters that were larger than the oysters used here.

In the second factorial design, water entering a portion of experimental array was prefiltered to about 5μ with a polypropylene filter bag. The filtered water was then remixed with unfiltered water to produce four filtered: unfiltered ratios - (100% filtered, 75% filtered, 50% filtered, 0% filtered). The four water types were maintained at three temperatures (11°C, 15°C, 20°C) to produce a filtration x temperature factorial design. Water flow was a constant 800 ml/min/20 oysters regardless of the filtered: unfiltered ratio.

The objective of the filtration experiment was to vary the food supplied to the oysters by reducing it without altering the water flow rate.

In order to measure the effectiveness of the filtration procedure we monitored the following parameters twice weekly: 1) Total organic carbon. The samples were preserved and taken to the Environmental Protection Agency laboratory in Corvallis. Total organic carbon was determined on 3 subsamples from each sample by Mr. Bill Griffis of the EPA using an Oceanography International, model 0524B Carbon Analyzer . 2) Particulate chlorophyll was determined using samples obtained concurrently with the carbon samples. Standard acetone extraction methods were used. The chlorophyll values were intended to provide an estimate of living phytoplankton.

Results - The carbon analysis and chlorophyll data are given in Table 12. These data show that the five-micron filtration reduced the organic carbon by only 15% while the chlorophyll was reduced by 51%. We can conclude from these data that most of the organic carbon in our water source was dissolved, colloidal, or of a particle size less than five microns. All of this organic carbon, even the dissolved materials, should be considered a potential food source for oysters. The chlorophyll data indicate, as might be expected, that chlorophyll is associated with particulate matter (Phytoplankton). The data further show that chlorophyll and therefore living phytoplankton did not constitute a large percentage of the total organic carbon in our water source. This experiment was conducted during a time of the year when phytoplankton densities could be expected to be low in Yaquina Bay. But, the fact is the oysters did show increases in meat weight (Table 13). This may mean that they were able to utilize organic carbon from sources other than living phytoplankton.

The growth results for the flow x temperature experiment (Table 13, Fig. 3) show a number of significant features. Unlike most of our previous studies, excellent meat growth was obtained at elevated temperatures. Keep in mind that we used water flow rates that on a per oyster weight basis were as much as eight times greater than any we had previously used. The leveling off of the growth curves of Fig. 3 indicates that our highest flow was approaching excess (i.e. further increases in flow would probably not have appreciably increased growth).

The best growth, both of meat (Table 13) and shell (Table 14), was obtained at 20°C, our highest temperature; but growth obtained at 20°C

Table 12. Newport Experiment III. Oct. 11-Dec. 15, 1973. Results of total organic carbon and chlorophyll a anlaysis on water samples drawn. Each value is the mean of three subsamples.

Date	15% filtration reduction		51% filtration reduction	
	unfiltered carbon (mg/l)	100% filtered carbon (mg/l)	unfiltered chlorophyll a (µg/l)	100% filtered chlorophyll a (µg/l)
11/01/73	1.70	1.40	2.27	0.97
11/06/73	2.60	1.90	2.12	0.82
11/07/73	2.05	1.40	2.04	0.97
11/11/73	2.83	2.13	2.94	1.40
11/16/73	3.47	2.93	1.86	1.19
11/19/73	2.00	2.07	1.19	0.59
11/21/73	1.83	2.23	1.54	0.64
11/27/73	1.97	1.73	1.48	0.84
11/28/73	2.07	1.63	1.72	0.88

Table 13. Newport Experiment III. Oct. 11-Dec. 15, 1973. Change in the mean wet weight (in grams) of shucked oyster meats as influenced by temperatures and flow. Values are means of 25 determinations. All water was unfiltered. Initial wet weight = 0.119 g.

Temp. °C	Flow (ml/min/25 oysters)			
	100	200	400	800
11	-.037	-.028	-.008	+.002
15	-.040	-.001	+.016	+.036
20	-.032	-.022	+.029	+.040

Table 14. Newport Experiment III. Oct. 11-Dec. 15, 1973. Percent increase in shell length as influenced by flow rate and temperature.

Temp. °C	Flow (ml/min/25 oysters)				
	100	200	400	800	$\bar{x}$
11	2.0	3.6	7.7	12.4	6.4
15	2.6	7.5	12.6	18.8	10.4
20	2.8	4.6	8.4	26.7	10.6
$\bar{x}$	2.5	5.2	9.6	19.3	

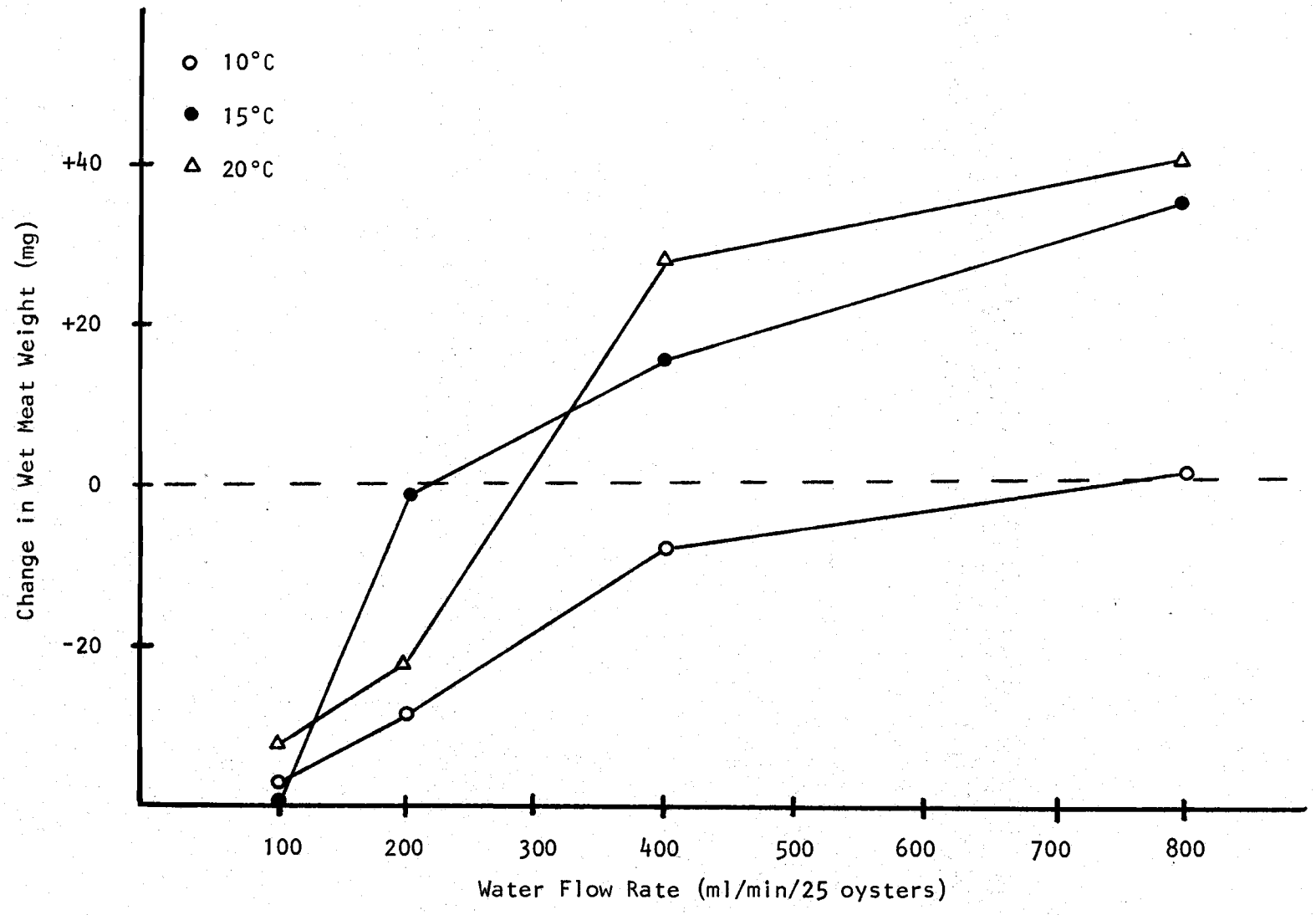

Figure 3. Relationship between water flow rate and the change in wet meat weight of oysters held at three temperatures. Newport Experiment III, Oct. 9-Dec. 5, 1973. Initial wet weight was 119 mg.

was not significantly greater than the growth observed at 15°C. In any case, there was a distinct growth enhancement at temperatures exceeding ambient.

It is significant to note that there were no mortalities in any of the treatments in this experiment. This is probably due to the increased flow rates (even our lowest flow was 4 times greater than our previous low flow), and to the relatively shorter duration of the experiment.

The filtration experiment did not show a systematic relationship between percent filtration and growth (Table 15). This may be because, as pointed out earlier, the filtration method did not remove very much of the total organic carbon.

Experiment IV - Newport May 15-July 17, 1974

Objective - The filtration experiment was discontinued after Experiment III. Experiment IV was conducted to provide flow rate x temperature x growth data during a time of the year when growth rate was expected to be quite high. The experimental array was also replicated to provide a growth and survival comparison between stocks of oysters obtained from two different commercial sources.

Design - The design of Experiment IV was essentially the same as Experiment III except that the filtration portion was omitted. Twenty-five oysters having an initial dry weight of 23 mg (the mean of a random sample of 50 sacrificed at the beginning of the experiment) were placed in each of 24 trays. Each tray was provided with seawater at one of twelve different flow x temperature combinations. The temperatures

Table 15. Newport Experiment III. Oct. 11-Dec. 15, 1973. Change in wet meat weight (in grams) of shucked oyster meats as influenced by percentage of 5 micron filtration of water supplied to the oysters at 800 ml/min/25 oysters. Initial wet weight = 0.119 g.

Temp. °C	Filtered 100%	Filtered 75%	Filtered 50%	Unfiltered	$\bar{x}$
11	+.027	+.024	+.022	+.007	+.020
15	+.046	+.013	+.007	+.052	+.030
20	+.004	+.025	+.036	+.059	+.031
$\bar{x}$	+.026	+.021	+.022	+.039	

used were ambient (about 10°C), 15°C, and 20°C, while the flows were 100, 400, 700, and 1,000 ml/min/25 oysters. The set of twelve was replicated so there were two of each of the treatments. Cultchless juvenile seed (length 23 mm) from the Bay Center Mariculture Co. were placed in one set of 12 trays. The duplicate set of twelve trays was stocked with juvenile oysters (length 20 mm) obtained from the Lummi Indian Oyster Hatchery.

It must be emphasized that it was not our intention to compare one commercial hatchery with another. Such a comparison would require a large number of sample lots and care to insure that the oysters were handled identically after shipment from the hatchery and prior to the start of the experiment. The results of this experiment in no way reflect on the quality of seed produced by either of the hatcheries. The experiment was designed only to provide a growth comparison between two different batches of oysters presumably having different handling backgrounds but subjected to identical experimental treatments.

Results - At the end of the experiment the surviving oysters were measured and shucked. The wet and dry weights of the meats were then determined and were compared with weighings made on initial samples of animals from the two oyster seed sources.

In this experiment we found no clear relationship between mortality and either temperature or water flow rate (Table 16). There was, however, a significant difference in the mortality experienced by oysters from the two seed sources. The Lummi stock showed a mean mortality of 29% among all treatments (a range of 16%-44%) while the Bay Center oysters experienced only a 3% mortality (a range of 0%-8%). At this point we

Table 16. Newport Experiment IV. May 15-July 17, 1974. Percent mortality among oysters subjected to 12 different temperature x flow combinations (L = Lummi Hatchery seed; BC = Bay Center Hatchery oyster seed).

Temp. °C	Flow (ml/min/25 oysters)									
	100		400		700		1,000		$\bar{x}$	
	L	BC	L	BC	L	BC	L	BC	L	BC
10	16	0	32	8	28	0	24	0	25	2
15	44	12	28	0	32	0	20	8	31	5
20	24	4	28	4	36	4	36	0	31	3
$\bar{x}$	28	5	29	4	32	1	27	3	29	3

Table 17. Newport Experiment IV. May 15-July 17, 1974. Percent increase in shell length among oysters subjected to 12 different temperature x flow combinations (L = Lummi Hatchery seed, BC = Bay Center Hatchery seed).

Temp. °C	Flow (ml/min/25 oysters)									
	100		400		700		1,000		$\bar{x}$	
	L	BC	L	BC	L	BC	L	BC	L	BC
10	37	40	109	78	85	97	97	100	82	79
15	66	37	103	86	92	129	132	102	98	89
20	30	35	52	48	120	84	116	122	80	72
$\bar{x}$	44	37	88	71	99	103	115	108	87	80

can only speculate as to the cause of the mortality difference between the two seed stocks. The important point is that there was a clear-cut difference in the survival of the two seed stocks although they were treated identically in the experiment. We recommend caution, therefore, in predicting seed survival under particular natural or experimental conditions unless those predictions are based on data obtained using seed from more than one source. This is particularly true if the prior handling and treatment history of the seed is not known.

The growth of the seed from the two sources was quite similar (Tables 17 and 18). The Lummi seed showed a mean increase in shell length of 87%, while the Bay Center seed increased in length by a mean of 80%. Moreover, as Table 17 shows, their responses to the flow-temperature combinations were similar.

Figures 4 and 5 show the influence of temperature and water flow on shell growth and on meat growth respectively. Both these Figures are for Lummi and Bay Center seed averaged together. As in previous experiments, there was a clear positive relationship between water flow and growth for all temperatures (Fig. 5). Unlike previous experiments, there was no weight loss; even the lowest flow rate provided sufficient food to maintain the animals' meat weight (Fig. 5) and even to provide for some shell growth (Fig. 4). The shapes of the growth curves further indicate that little advantage in shell growth could be obtained by increasing the water flow from 700 ml/min to 1,000 ml/min (28 ml/min/oyster - 40 ml/min/oyster). Meat growth, on the other hand, showed no such leveling off at the 700 ml/min flow rate (Fig. 5). Unlike any previous experiment, the curves (Fig. 5) indicate that maximum meat growth would

Table 18. Newport Experiment IV. May 15-July 17, 1974. Increase in dry meat weight in mg among oysters subjected to 12 temperature x flow combinations (L = Lummi Hatchery seed, BC = Bay Center Hatchery seed).

Temp. °C	Flow (ml/min/25 oysters)									
	100		400		700		1,000		$\bar{x}$	
	L	BC	L	BC	L	BC	L	BC	L	BC
10	31	12	110	62	96	125	124	149	90	87
15	29	21	89	95	200	117	235	220	138	113
20	13	15	51	58	141	112	-	248	-	108
$\bar{x}$	24	16	83	72	146	118	-	206		

Table 19. Newport Experiment IV. May 15-July 17, 1974. Total organic carbon and chlorophyll a data taken from unfiltered incoming seawater. Each value is the mean of 3 replicate subsamples taken from a 24 hr composite sample preserved with mercuric chloride.

Date	Total organic carbon (mg/l)	Chlorophyll a (µg/l)
05-16-74	1.70	4.55
05-20-74	2.50	4.63
05-26-74	2.13	3.35
06-05-74	1.27	2.69
06-25-74	1.93	1.87
$\bar{x}$	1.84	3.42

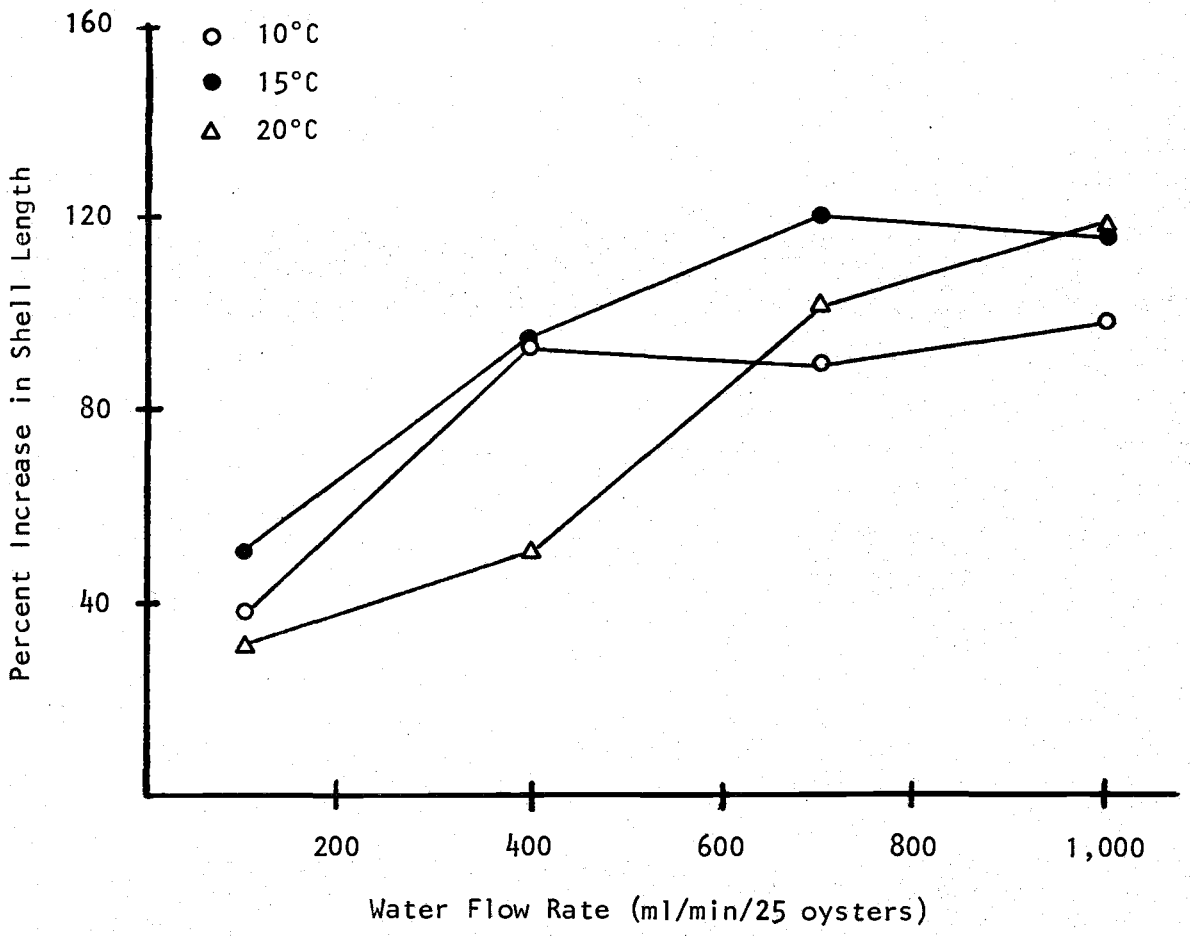

Figure 4. Percent increase in shell length (means of Lummi and Bay Center lots) of oysters subjected to 12 combinations of temperature and water flow rate. Experiment IV May 15-July 17, 1974.

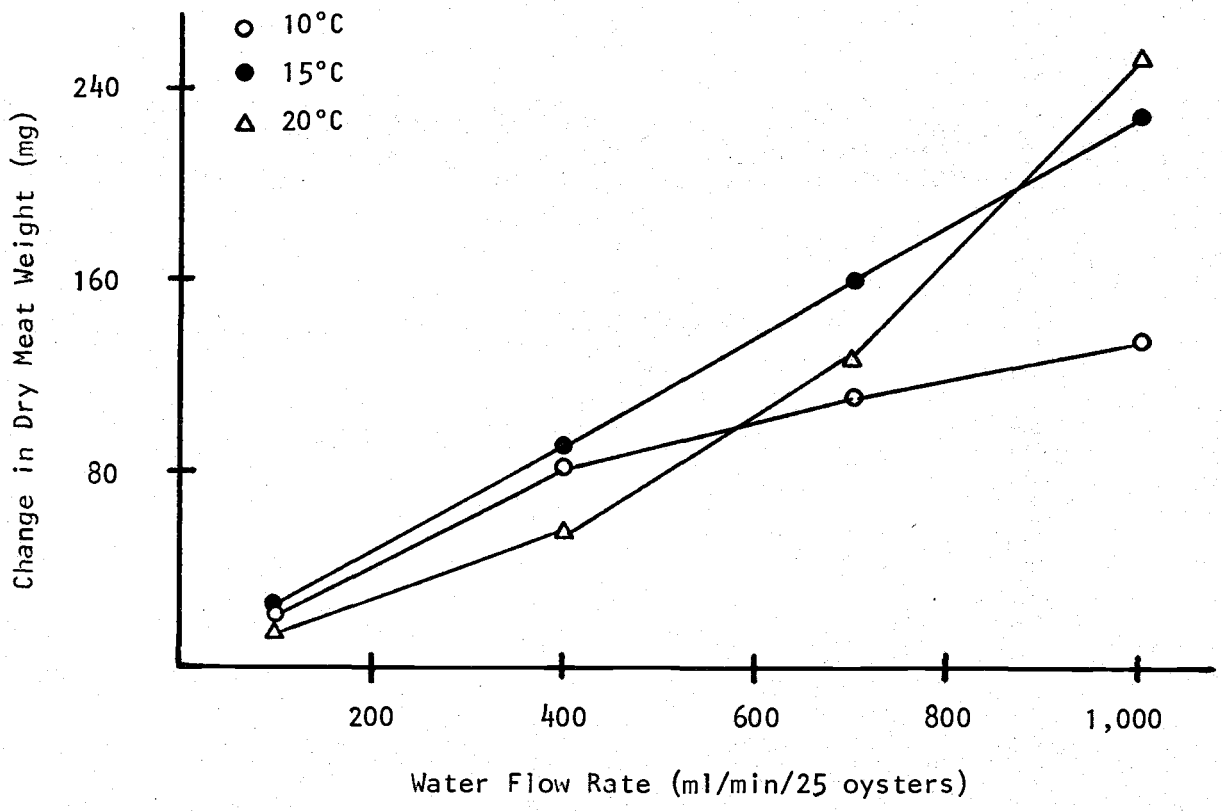

Figure 5. Increase in dry meat weight for oysters held at three temperatures and four flow rates, Experiment IV, May 15-July 17, 1974. Initial dry meat weight was 23.1 mg. Plotted values are means of Lummi and Bay Center seed.

probably be obtained at some flow rate exceeding 40 ml/min/oyster (1,000 ml/min in this experiment).

Seasonal Variation in Oyster Growth

One very obvious feature among the previously described oyster growth experiments is that, although the general relationships remained relatively constant, the absolute value of growth obtained for any given treatment varied greatly with season. This feature is made clearer in Table 20 which shows the rates of shell growth observed in oysters held at 15°C and provided with a flow of 8 ml/min/oyster at the Marine Science Center in Newport. Note that more than a ten-fold range in shell growth rate (0.12-1.50) was observed. Table 20 also gives mean total organic carbon (TOC) and chlorophyll a values for the duration of each experiment. The significance of these determinations will be discussed in a later section.

Although seasonal growth differences among the four growth experiments in Table 20 are obvious these experiments were not intended to provide a complete profile of seasonal variation in oyster growth. Other, long term, experiments were carried out at the Port Orford laboratory in 1971-1972, and in Newport in 1974-1975, to provide a clearer picture of the kind of seasonal fluctuations in oyster growth that might be expected.

The Port Orford experiments of 1971-1972 were of relatively small scale. These experiments were conducted using oyster spat attached to shell cultch. Cultch pieces as well as individual spat were numbered so that repeated measurements could be made on the same spat. Fifteen individuals were measured for each data point. The animals were provided

Table 20. Shell growth observed in oysters of similar size during different times of the year. Growth in all four experiments is for animals held at 15°C with a water flow rate of 8 ml/min/animal. All four were conducted at the Marine Science Center, Newport.

Experiment number	Dates	Duration (days)	Initial shell length (mm)	Shell growth (% per day)
I	Jan. 30 - March 18	54	25	0.17
II	March 30 - June 22	84	25	0.30
III	Oct. 9 - Dec. 5	57	21	0.13
IV	May 15 - July 17	63	23	1.50

with one l/min of open coast seawater. The results of the experiment are shown in Figure 6.

The Newport experiment of 1974-1975 was a large scale experiment which employed a 15,000 gallon outdoor tank stocked with about 4,400 spat c shell cultch. The tank was supplied with 10 gpm of ambient temperature Yaquina Bay water. A random sample of 750 spat was measured for each data point in Figure 6. Note that two different groups of oysters were tested simultaneously in this experiment. One group had an initial shell length of 2.0 mm, while the larger group averaged 8.9 mm in length.

A number of important characteristics of the observed fluctuations in the growth rate of oysters are shown in Figure 6. Notice for example that there is a period of time between October and April during which little or no growth occured. Among the smaller Newport oysters, for example, only 14% of the growth observed during a one year period occured between the months of October and March. Note, further, that this low growth period appears to be independant of temperature. One group of Port Orford oysters (shown on Figure 6) showed the same leveling off of growth during the fall as did the ambient temperature group. Additionally, as Table 20 shows, growth experiments conducted during the winter months (Experiment I and Experiment III) yielded much less growth than the spring and summer experiments (II and IV) despite the fact that the temperature was held at 15°C in all four experiments. Finally, Figure 6 indicates that the October to April growth slowdown is relatively independent of the animal's size at least within the 8-40 mm size range. Similarly, growth rate showed a spring recovery in all the size classes (Fig. 6).

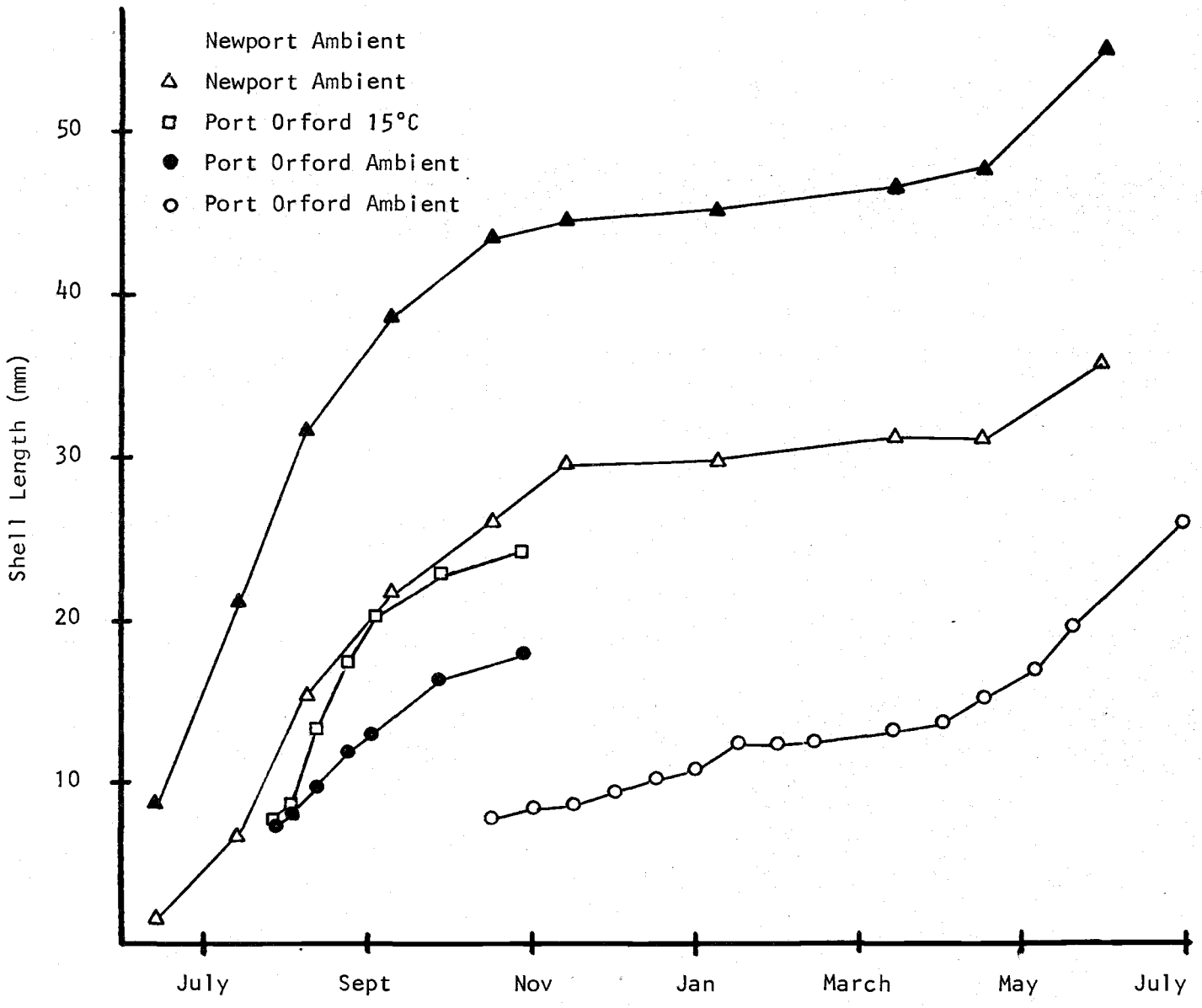

Figure 6. Change in shell length by oysters held in Newport (Yaquina Bay at the Marine Science Center) and Port Orford Oregon. The curves show periods of rapid increase, particularly in the spring and a period from about mid-October to April during which little or no growth occurs.

Yaquina Bay experiences considerable fluctuations in salinity during the winter months, and it is possible that periods of low salinity, interfering with normal feeding, may have contributed to the reduction in oyster growth observed during those months. However, the open coastal location at Port Orford did not experience such radical salinity fluctuations during the winter, and yet oyster growth essentially ceased after October.

In conjunction with the Newport experiments, total organic carbon (TOC) and chlorophyll a determinations were periodically made for several months to ascertain how these parameters varied with season. We hypothesized that observed changes in oyster growth were caused primarily by seasonal changes in the quantity of food available to the aniamls. By monitoring TOC and chlorophyll a we hoped to quantify those expected fluctuations. The results of these determinations indicate that TOC is not a good measure of food material available to oysters. The mean of 35 determinations made between Nov. 1 and April 1 a period of very little oyster growth (Fig. 6) was 1.92 mg/l. The mean of 25 determinations made between April 1 and July 1, a period of rapid growth in oysters was 1.74 mg/l. It is possible that the relatively high organic carbon values obtained during the winter months were caused by the addition of organic detritus to the estuary by rainfall runoff. In any case it appears that much of the organic material present in the estuary (a significant precentage of which is dissolved in the water) is not suitable food for oysters.

Many of the chlorophyll values obtained during this study were lower than those that have been reported in other studies. This may be

due to the method of sampling whereby a 24 hour sample was continuously pumped from the bay and dripped at a very slow flow rate into a large container. Sub-samples were then taken from this sample for chlorophyll determination. In any case chlorophyll a values are notor ously variable and are very difficult to relate in an absolute way to a specific quantity of phytoplankton. Nevertheless, the relative magnitudes of values we obtained do seem to be related to observed growth in oysters. The mean of 14 samples (three determinations made on each sample) taken during the period of low oyster growth, Oct 1 - April 1 is 1.57 µg chlorophyll a/l (a range of 0.83-2.94). The mean of 8 samples taken in the Spring (April 1 - June 25), a period of very rapid oyster growth is 3.42 µg chlorophyll a/l (a range of 1.64-5.50).

In general we have concluded that TOC is of little or no value as an indicator of potential oyster gorwth. Chlorophyll a, although grossly related to oyster growth, is subject to considerable variation due to variation in the actual quantity of phytoplankton present, to the patchy distribution of this material, and to variation in the quantity of chlorophyll present in any given mass of phytoplankton. Further, it is still unclear how important living plant material is as food for oysters, compared for example to ditritus, bacteria, or other organic material. Consequently, although chlorophyll a concentration may be relateable to oyster growth after the fact, at present it has no predictive value.

Closed System Studies

System Design

During the winter of 1974-1975 a 1,000 l recirculating system was constructed and tested at OSU's Marine Science Center. The purpose of the system is to provide a precisely controlled environment for studying the food consumption and growth of oysters at different temperatures. The system provides flowing seawater at four different temperatures from a common treatment system. The water treatment consists of coarse filtration (down to 44 microns), a foam column for removal of some dissolved organics, biological filtration through dolomite gravel, fine filtration (down to 0.8 microns), carbon filtration, U.V. sterilization, and finally, temperature regulation. Any type of diet, mainly cultured algae at this point, can then be metered into the water as it enters the oyster holding trays. There are 16 of these trays (four at each temperature) so that as many as four ration levels can be tested at each temperature. Since the water recirculates, environmental fluctuations (salinity, pH, etc.) are avoided. Further, since there is a single water treatment system, changes in water quality are the same for all treatments.

Objective

Studies with the closed system have two general objectives. Our primary objective is to use the closed system to determine how the energy content of consumed food is budgeted by oysters to waste products, oxygen consumption, and growth at various temperatures and levels of

food availability. This information, then, will tell us how growth is affected by temperature and food supply, and will provide the basis for a greater understanding of the growth responses that we observed in previously described experiments.

A second objective of the closed system studies is to obtain the background data necessary to evaluate the feasibility of providing supplemental food to cultured oysters during periods of low natural food availability. Data generated by these studies will provide a basis for selecting optimum food concentrations, for estimating food requirements, and for predicting oyster growth rates. Studies have begun using cultured algae as oyster food, but it is unlikely algae could be used to supplement oyster food on a commercial scale. Therefore, later studies will consider alternative food types, including both particulate and dissolved materials.

Preliminary Closed System Experiments

The first experiment in the closed system was initiated in August of 1975. The temperatures used were 11°, 15°, 19°, and 23°C. The oysters were provided with 4, 2, 1, and 0 ml/min of stock culture of the planktonic flagellate Platymonas suecica having a concentration of 500,000 cells/ml. Since the above algae dripped into water flows of 400 ml/min, algae concentrations of 5,168, 2,584, 1,292 and 0 cells/ml entered the oyster trays. Carbon analysis done on samples of algae prior to the start of the experiment showed that the algae contained about 7.7×10^5 µg carbon/cell. So the cell concentrations used correspond to 400, 200, 100 and 0 µg particulate carbon/l. Fifty oysters were

placed in each of the sixteen trays at the beginning of the experiment.

Within two weeks measurable food consumption ceased in all of the treatments, and by the end of the third week 100% mortality had occurred among oysters held at 23°C. About 65% mortality occured in the 19°C trays, 2% in the 15°C trays, and 0% in the 11°C trays. The experiment was terminated at the end of the third week.

Analyses carried out by members of OSU's Microbiology Department showed high bacterial concentrations in the water. Further, a variety of bacterial species were identified from the shell cavity of the oysters. Among those identified were one of more vibrios, a genus which includes some species thought to be pathogenic for oysters.

It was painfully obvious, then, that disease would be a serious problem in closed system experiments, particularly at high temperatures. Additional precautions were taken in subsequent experiments to minimize these disease problems. The same precautions that we have employed in our small scale experiments to control diseases would very likely also be necessary in large scale culture where supplemental feeding is used.

In commercial culture operations disease is generally avoided by (1) minimizing stress on the cultured animal (i.e. avoidance of crowding, temperature control, etc.), and (2) creating conditions that are not favorable for the growth of disease agents (again, temperature control, avoidance of excess feeding, keeping culture vessels clean, etc.). In extreme cases antibiotics or vaccination may be employed. Since our experimental work in a closed system unavoidably involved stress on some of the animals (e.g. high temperature), we concentrated our efforts

on preventing the growth of disease agents in the culture system. This was done by (1) cleaning the culture trays and washing the oysters every 48 hours to prevent the buildup of feces and detritus, (2) checking for and removing mortalities twice daily, and (3) improving water filtration system to minimize background levels of organics in the water and on the filters. These measures held mortalities to an average of 21% in our second closed system experiment. We consider 21% to be an acceptable mortality level considering that it includes mortalities among animals that were held for seven weeks with no food at temperatures as high as 23°C.

Although disease seems to be under control in the closed system, we have not yet obtained appreciable growth among juvenile oysters fed cultured algae. Since the quantity of food provided on an organic carbon basis was more than adequate, and since the oysters did consume large quantities of the algae, we believe that a diet of a single species of cultured algae may be qualitatively inadequate. Future experiments will involve the use of at least two food species fed on alternate days.

In this way we hope to provide a more balanced diet. Studies using this closed system to meet objectives previously discussed will continue with support from the National Sea Grant Program.

Discussion and Conclusions - Oyster Growth Experiments

An obvious feature of our oyster growth studies is that no two experiments gave the same results. Those experiments (I-IV) were intended primarily to provide data that would permit us to define water

flow requirements for a given quantity of oysters at a given temperature. Obviously, the situation is not that simple. In the first place, of course, oysters do not eat water, but rather they require a flow of water as a source of dissolved oxygen, to dilute and carry away wastes, and as a food containing and distributing medium. Our studies indicate that the quantity of food contained in a volumn of seawater varies with season. Since there is, therefore, no simple relationship between water flow and oyster growth that is valid for the entire year, it is not surprising that Experiments I-IV yielded such varied results. Further, since it has not yet been determined with certainty exactly what oysters eat, attempts to determine the food content of a given water source by direct measurement have met with limited success.

Our experiments have served to assay in a relative sense the capacity of a water source to support the growth of oysters. These experiments have also shown that significant growth advantage can be obtained by heating seawater to about 15°C compared to ambient temperatures of about 10°C. We found, however, that there was generally little or not advantage to a culture temperature of 20°C compared to 15°C, also since mortality has proven to be a more serious problem at 20°C than at 15°C, we feel that an optimum culture temperature for long term growth is close to 15°C.

SALMON GROWTH STUDIES

Introduction

Intensive culture of salmonids in raceways and ponds is an established industry in freshwater. Similar culture techniques are now being applied on a large scale to a saltwater environment by Oregon-Aqua Foods, Inc. in Newport, Oregon. Although intensive salmonid culture in saltwater is still in its early stages of development, its initial successes are encouraging and expansion is likely. Current commercial emphasis is on coho salmon, Oncorhynchus kisutch and rainbow trout (steelhead), Salmo gairdneri.

Our studies of the feasibility of culturing salmon in seawater heated with the effluent of nuclear power plants concentrated on the chum salmon O. keta, and to a lesser extent on the pink salmon O. gorbuscha. This was done because these species, unlike other salmon, adapt to salt water as fry and would require only a minimal fresh water facility. Moreover, the Oregon legislature removed legal restrictions on commercial chum salmon hatcheries in 1971, and thus permitted private chum hatcheries to operate in Oregon under license to the Oregon Department of Fish and Wildlife (formerly Fish Commission of Oregon). Such hatcheries could provide a private source of seed stock for large scale intensive culture. More recently, restrictions on private coho and chinook hatcheries have also been eased somewhat, but the number of private hatchery licenses being issued for coho and chinook is still very limited.

Preliminary Studies

Preliminary work with pink and chum salmon was completed in the summer of 1972. The results of these studies have been described in previous progress reports. The work was done primarily at the Port Orford Laboratory by Bernard Kepshire and was the subject of his PhD dissertation (Department of Fisheries and Wildlife, Oregon State University, Oct. 30, 1975). These studies are summarized below.

Objectives

The major objective of our preliminary studies was to determine the temperature at which the highest growth rates for chum salmon, Oncorhynchus keta, and pink salmon, O. gorbuscha, occur when they are fed to satiation. Food consumption rate, gross food conversion efficiency, and survival were also determined.

Methods

Both species of salmon were reared in rectangular 100 gal tanks supplied with 0.5 gpm of seawater. Immersion heaters were used to maintain temperatures of 13°, 15°, 18°, and 21°C in the tanks. Water temperature, salinity, and dissolved oxygen were monitored. The fish were fed Oregon Moist Pellet (OMP) of a diameter appropriate for the size of the fish. Fish were fed twice daily, at 0900 and 1700 hr. During each feeding period the fish were fed all that they would consume in one hour. The growth rate of each treatment group was deter-

mined every tenth day. All the fish in each group were anesthesized singly or in groups (depending on their size), placed on a dry towel to remove excess moisture, and weighed to the nearest 0.01 g. The fish were placed in an auxilliary tank until all from a group had been weighed. They were then returned to the rearing tank.

Food consumption rate was determined by weighing the amount of food added to each tank and subtracting from that weight the calculated weight of uneaten pellets siphoned from the tanks after the feeding period. Food consumption was expressed as percent of body weight/day (wet weights). Gross food conversion efficiency was then determined as the gain in fish weight (total wet weight/group in grams) divided by food consumed (wet weight, grams) times 100%.

Results

For chum salmon the highest food consumption rate occurred at 18°C; highest gross food conversion efficiency occurred at 13°C; greatest growth occurred at 15°-18°C; and greatest survival occurred at 13°- 15°C. The response in the parameters measured was poorest for both chum and pink salmon at 21°C. Pink salmon generally had a higher food consumption rate, gross food conversion efficiency, and growth rate than chum salmon.

Weight specific food consumption, gross food conversion efficiency, and growth rate of chum and pink salmon declined as body weight increased. In general these parameters declined more with increasing body weight at higher temperatures than at lower temperatures (13°-15°C) for both chum and pink salmon.

Disease

Bacterial Kidney Disease was found to be prevelent in a number of the experimental groups of both chum and pink salmon. Bacterial Kidney Disease (BKD) is contracted by fish in fresh water and is caused by a species of Corynebacterium. Since BKD is generally a chronic disease, it did not cause high mortalities, but probably affected determination of food consumption, conversion, and growth. Vibriosis, which proved to be a serious problem in subsequent experiments carried out at Newport, was virtually non-existant in these experiments.

Temperature x Ration Experiments

Our preliminary studies indicated a need for additional work in two general areas, (1) disease control, and (2) the effects of different ration levels on the food consumption and growth of cultured salmon at various temperatures. A number of experiments were designed and conducted through the winter and spring of 1973 to provide additional information concerning these two problem areas.

In the experiments described in the following section, the fish used were chum salmon reared from eggs taken in the fall of 1972 at the Oregon State University hatchery on Netarts Bay. The work was done by Mr. Gerald Rowan and is described in additional detail in his master's thesis (Department of Fisheries and Wildlife, Oregon State University, June 1975).

In our preliminary studies we determined the growth rates of pink and chum salmon juveniles at four temperatures, but at a single food

ration level. Studies conducted in Newport during 1973 were designed to determine the combined effects of ration level and temperature on the food conversion and growth of chum salmon.

Fish used in this study were vaccinated against Vibrio following procedures to be described later. A pooled group of about 10,000 fish was held in a number of large ambient temperature tanks at the Marine Science Center in Newport. Fish were drawn at random from these tanks for use in the following experiments.

Experiment I - May 29-July 7, 1973

Design - Fish were reared in 16 temperature-controlled tanks such that there was one tank at each combination of four temperatures (11°C, 14°C, 17°C, 20°C) and four ration levels (3%, 6%, 9% and 15% of body weight per fish per day). Following a short acclimation period the experiment was continued for 40 days. At 10-day intervals during the experiment twenty fish from each treatment were randomly selected for weighing. The fish weighed about 1.3 g (wet) at the beginning of the experiment and had a maximum wet weight of about 2.2 g at the end.

Results - Growth rates were found generally to be higher at lower temperatures for a given ration level (Fig. 7). The difference in growth rate between fish held at 11°C and those held at 14°C is certainly not great. The significant point here is that there appeared to be no growth advantage at the higher temperature.

Figure shows quite clearly the metabolic costs to the fish of increased temperature. At 20°C a ration level of 15% of dry body

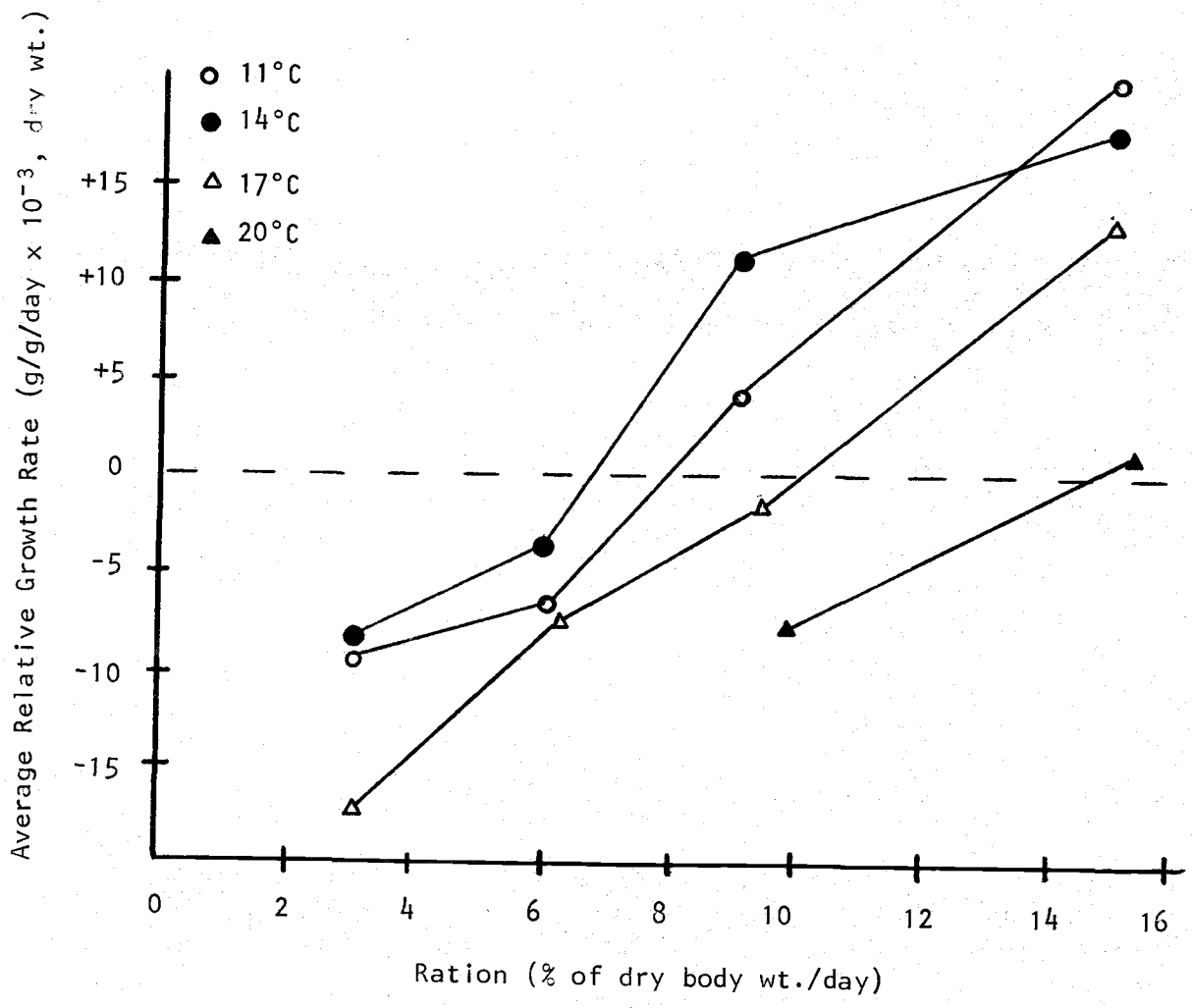

Figure 7. The relationship between ration and growth rate for chum salmon held at four temperatures. Experiment I, May 29-July 7, 1973. The fish weighed about 1.3 g at the beginning of the experiment and about 2.2 g at its termination.

weight per day would have been required to just maintain the fish, that is, to prevent either gain or loss of weight. At 17°C the maintenance ration was about 10%, and it dropped to 6-7% for the two lower temperatures.

Of the four temperatures tested, the temperature yielding the best growth seems to be about 14°C for most ration levels (Fig. 8). That temperature is only slightly above ambient for Newport during the summer months.

Mortality from Vibrio was found to be directly related to temperature and inversely to ration (Fig. 9). These data provide a classic example of the influence of stress on disease incidence among cultured animals. In this case stress, as inadequate ration, excessively high temperature, or particularly as a combination of the two, produced high mortalities due to Vibrio.

Food conversion efficiency (a measure of the percentage of food consumed appearing as an increase in weight) was found to be directly related to ration level and inversely related to temperature. Since only the fraction of the food consumed that is in excess of the maintenance requirements is available for growth, the low conversion efficiencies at higher temperature reflect the high maintenance requirements at those temperatures (shown in Fig. 7). As with growth, the best food conversions were found at the highest ration (15%) and lowest temperature (11°C) and at the two higher rations (9%, 15%) at 14°C. Conversion efficiencies at 17° and 20°C were low.

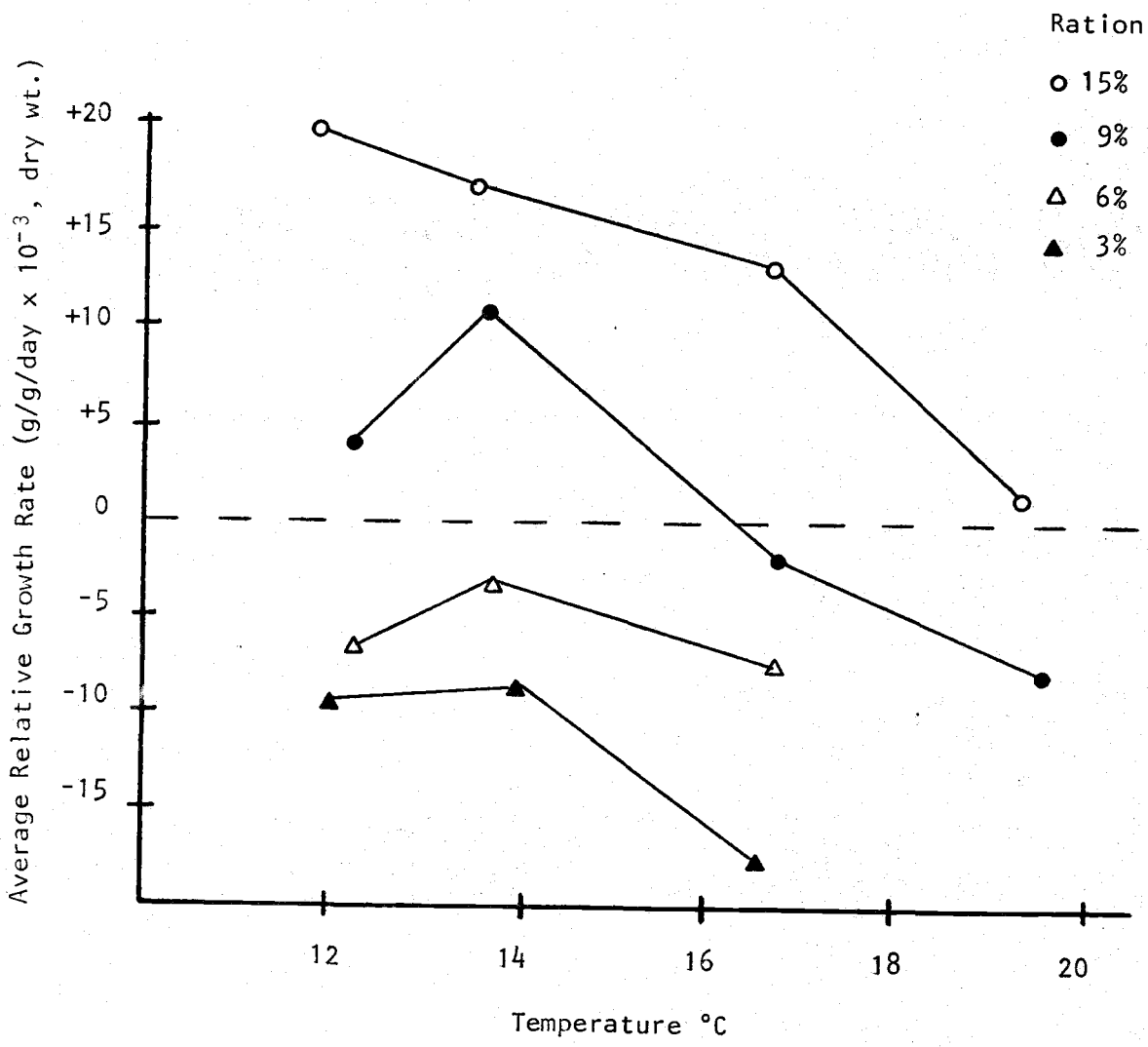

Figure 8. The relationship between temperature and growth rate for chum salmon fed at four different ration levels (as % dry body wt./day). Experiment I, May 29-July 7, 1973.

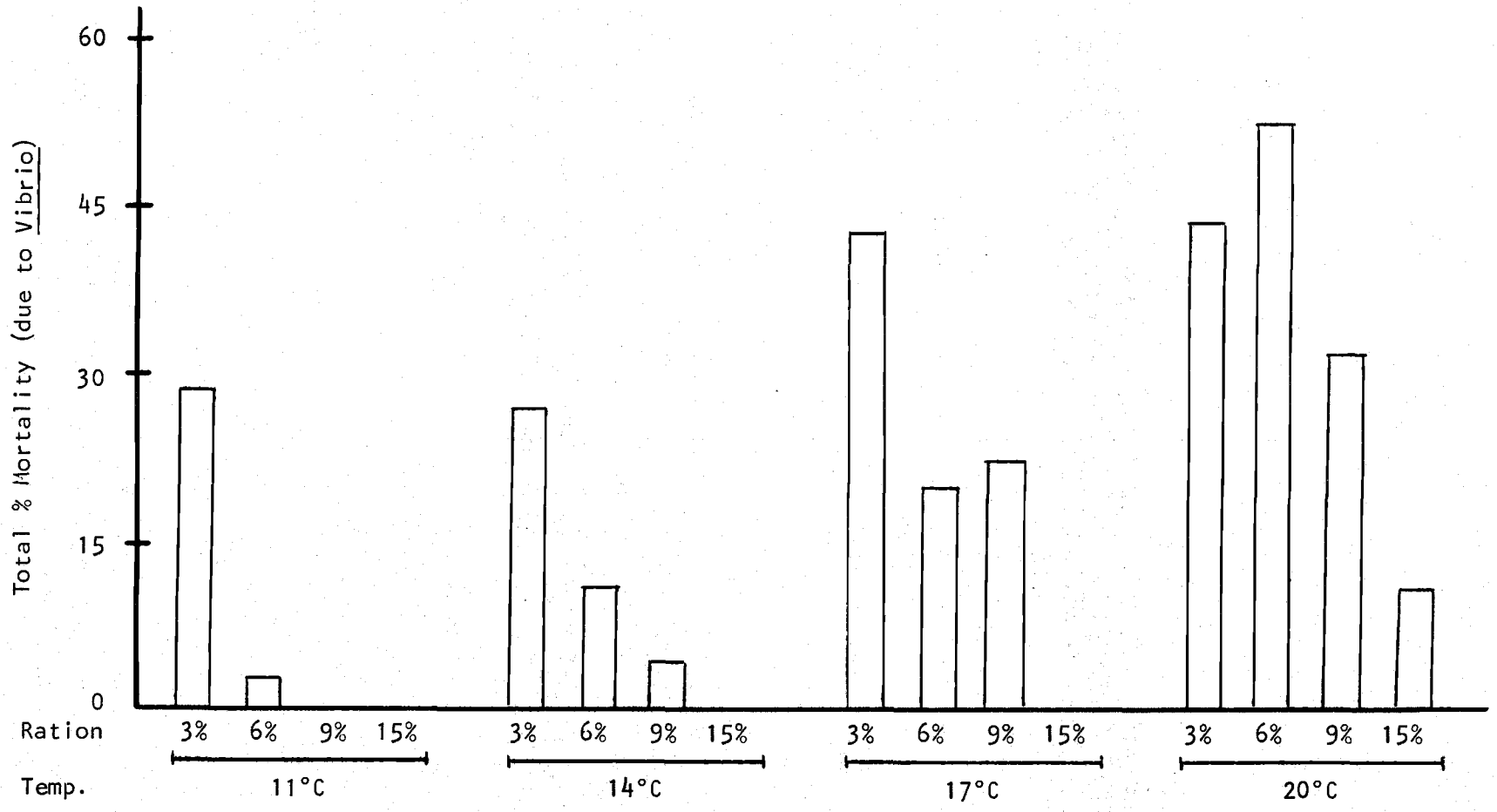

Figure 9. The combined influence of temperature and ration level (per cent of dry body weight per day) on mortality due to Vibrio in chum salmon. Experiment I, May 29-July 7, 1973.

Experiment II - Sept. 18-Nov. 7, 1973

Design - The second experiment of 1973 was conducted following the same general design as the first experiment. Minor changes were made in the sampling procedure and ration levels were adjusted to 3%, 8%, 13% and 18% of dry body weight per day. Fish used in the experiment averaged about 12 g wet weight at the beginning and had a maximum wet weight of about 40 g at the end.

Results - The results of Experiment II were similar in most respects to those of the first experiment.

As in the first experiment, the data from Experiment II show that the best growth was obtained from our highest ration (Fig. 10). In Experiment II, however, we found that the maximum food consumption by the fish, as a percentage of body weight, was somewhat less than Experiment I. We also found that the maintenance ration as % of body weight (ration permitting zero weight change) was less in Experiment II than in Experiment I for all temperatures. These differences in consumption and maintenance are attributable to the larger size of the fish used in the second experiment. It is generally true that fish consume and require a lower fraction of their body weight in food as they become larger.

The best growth was recorded at the higher ration levels and at about 14°C (Fig. 11). The growth curves for the two higher ration levels are quite similar (Fig. 11). This is because the fish never actually consumed more than about 15% of their body weight. Therefore, on the basis of food consumed the two higher ration levels (nominally 13% and 18%) were nearly identical.

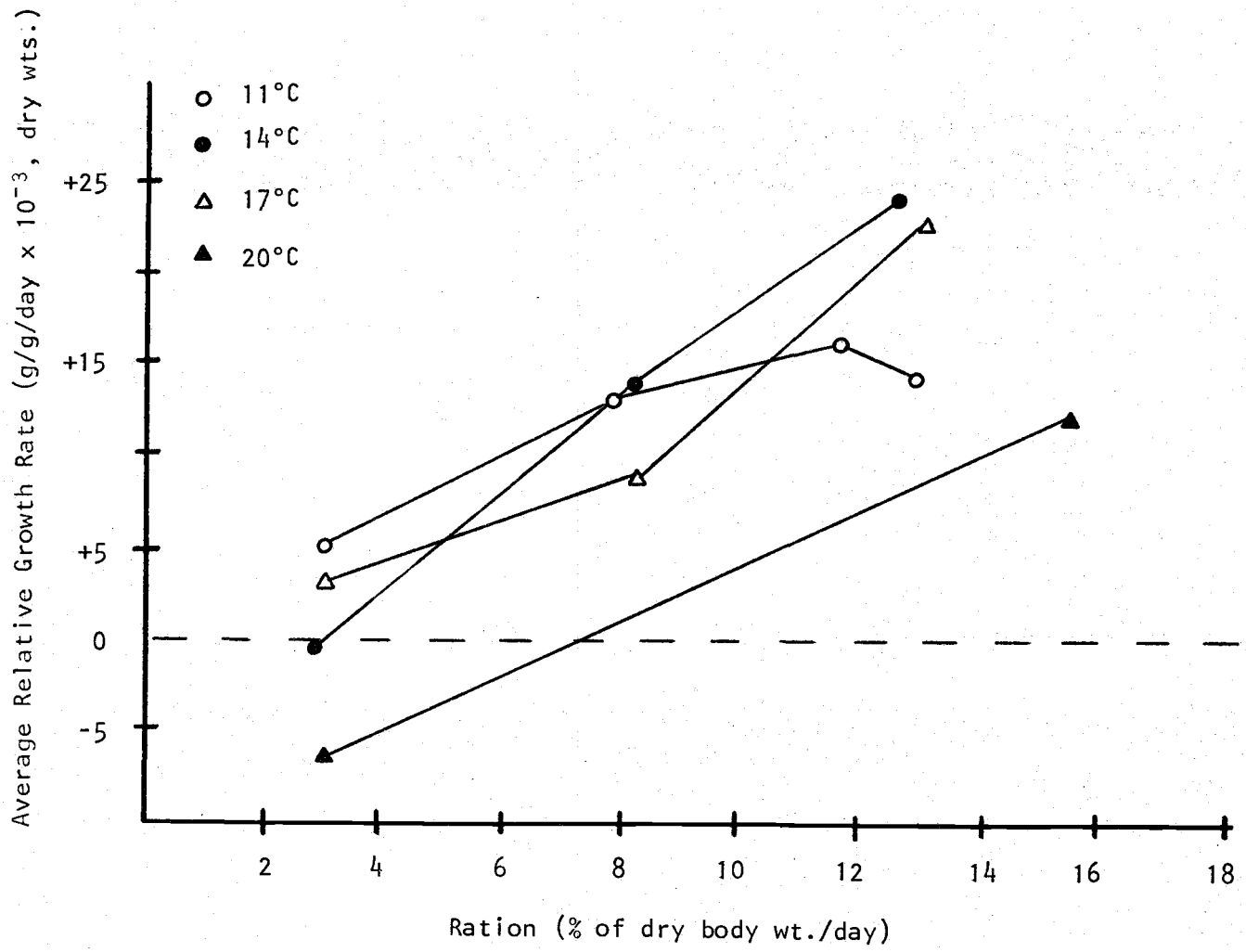

Figure 10. The relationship between ration level and growth rate for chum salmon held at four different temperatures. Experiment II, Sept. 18-Nov. 7, 1973. The fish weighed about 12.4 g at the beginning of the experiment and about 18.4 g at its termination.

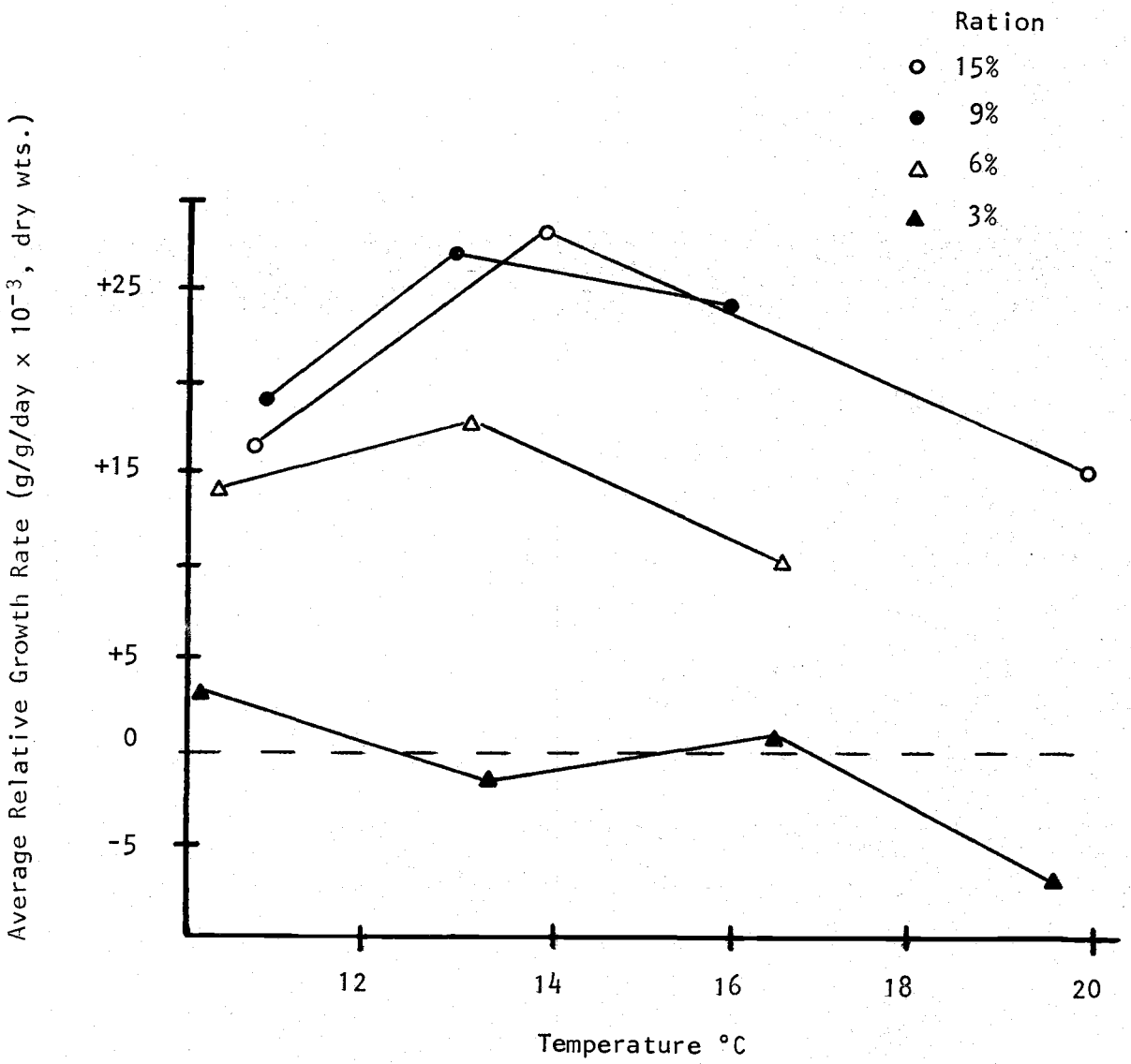

Figure 11. The relationship between temperature and growth rate for chum salmon fed at four different ration levels (as % dry body wt./day). Experiment II, Sept. 18-Nov. 7, 1973.

Mortalities in Experiment II were generally higher at the higher temperatures totalling 6%, 23%, 40%, and 36% at 11°, 14°, 17° and 20°C respectively. Unlike Experiment I, mortalities did not show a clear relationship with ration level in this experiment. This may be because even the lower rations were in excess of maintenance for all but the higher temperature groups (compare Figs. 7 and 10) so that low ration was a less significant stress factor in Experiment II than in Experiment I.

Disease Control

Disease control efforts during this series of experiments were concerned basically with (1) identifying the types and extent of disease problems that we might encounter, and (2) preliminary efforts to control disease.

Bacterial Kidney Disease

In our earlier work we encountered a serious problem with Bacterial Kidney Disease. This type of kidney disease is caused by fresh water bacteria (Corynebacterium sp.) and has no effective treatment. The disease is chronic but can cause mortalities at elevated temperatures.

Since the disease is of fresh water origin, we theorized that it could be avoided by keeping the fish in a disease-free environment during their short fresh water period.

To test the hypothesis we split a group of chum eggs into two experimental lots, one of which was incubated following standard

hatchery procedures using untreated water from Whiskey Creek, while the other was "sterile incubated". Sterile incubation consisted of the following treatments: 1) sterilization of the eggs with a bath of Wescodyne, 2) sterilization of the incubators with chlorine prior to use, and most importantly 3) continuous sterilization of the water supply (de-chlorinated city water) with ultra-violet light.

After these fish hatched and had "buttoned-up" they were moved to salt water rearing tanks at the Marine Science Center in Newport. The groups were kept separate and had subgroups at ambient (11°C) and elevated (17°C) temperatures.

The results of the study were evaluated on the basis of the comparative growth and mortality of the groups, and on examination of fish for signs of disease and disease agents.

Since the experiment was compounded with an experimental vaccination program to control Vibriosis, the results will be discussed following discussion of the vaccination experiment.

Vibrio

Vibriosis, caused primarily by the marine bacterium Vibrio anguillarum, was known to be a serious problem in saltwater fish culture. We had not had real problems from Vibrio before 1973, but the experience of others suggested that we should expect difficulties as we scaled-up our work in Newport.

In an attempt to minimize expected problems from Vibrio we carried out an experimental vaccination program. The work was done primarily by Dr. John Fryer and Mr. Dave Ransom of O.S.U.'s Microbiology Depart-

ment. Vaccination consisted of feeding the fish killed bacterial cells mixed in with prepared fish food. The vaccine was, in this case, mixed with the starter mash and fed to the fish as soon as they started to feed. All of the fish intended for use in the growth studies were vaccinated. Sub-groups from the kidney-disease experiment (previously described) were also vaccinated. The final design of that experiment is as shown in Figure 12.

Results - High mortalities were recorded among all of the experimental groups shown in Figure 12. In fact, essentially all of the fish used in that aspect of the disease work died before the end of the summer. Vibrio was the only pathogen isolated from these fish.

Since no Corynebacteria were found and because none of the mortalities are attributable to kidney disease, it is impossible to evaluate the effectiveness of the sterile rearing procedure. Further, since the mortalities among even the vaccinated fish in this experiment eventually amounted to 100%, it must be said that the vaccination provided at best only limited protection from Vibrio. However, the sequence in which total mortality occurred (Fig. 12) indicates to us that the treatments may have had some effect on the fishes' susceptibility to Vibrio.

Notice (in Fig. 12) that the unvaccinated fish held at 17°C were the first two groups to show 100% mortality. Then, consider the four groups of fish held at 11°C as two pairs, each of which contained a vaccinated and an unvaccinated group. Notice that, as a pair, the sterile incubated groups survived longer than the unsterile group, and

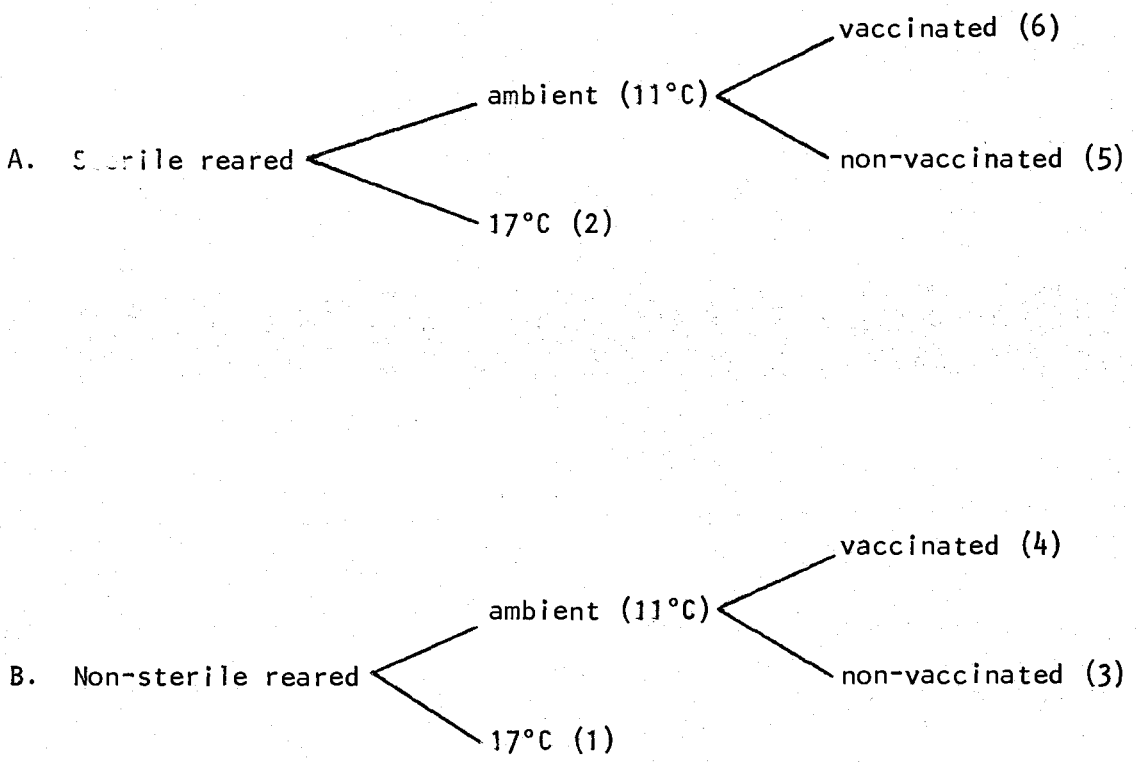

Figure 12. Design of kidney disease and Vibrio experiment conducted with chum salmon in Newport, 1973. Sterile rearing (in fresh water) was a kidney disease control measure, while vaccination (in saltwater) was to control Vibrio. Numbers in parentheses indicate sequence in which 100% mortality was observed.

within each pair the vaccinated group showed better survival than the unvaccinated group.

Certainly this experiment does not establish conclusively that vaccination affords protection against Vibrio. That was not its objective. But, it does provide us with sufficient encouragement that we feel the technique warrants further study.

There are a number of possible reasons why our vaccination procedures failed to provide complete immunity from Vibrio. We attempted to mix the vaccine with "starter mash" so that we could feed it to the fish soon after they entered saltwater. This deviates from the standard procedure in which vaccine is mixed with larger pellets. Mash is not only more difficult to work with simply because of its physical nature, but fish that are that young feed poorly and waste much of the food. It was, therefore, almost impossible for us to get a good estimate of the amount of vaccine actually consumed by the fish.

Further, it is the nature of the immune response that a challenge by Vibrio organisms is necessary to maintain the immunity. We feel now that the fluctuations in environmental conditions in our water source (Yaquina Bay) cuased intermittent, rather than constant, challenges by Vibrio. The degree of immunity in the fish may have been lowered by a period during which Vibrio was not present in large numbers. Then as the environment changed, high numbers of Vibrio could have challenged the fish when their immunity was at a low level. Finally, we cannot exclude the possibility that chums are immunologically incompetent.

Based on evidence that we have already discussed, it seems that stress was the most important factor in determining resistance to Vibrio. Stress of any kind (temperature, inadequate food, fluctuations in

salinity, etc.) invariably contributed to high mortalities in spite of our efforts to protect the fish by vaccination and occasionally by the use of antibiotics.

Discussion

None of the experiments conducted between 1971 and 1974 indicate any advantage in culturing chum salmon at temperatures in excess of 14°C. Nevertheless, since ambient seawater temperatures on the Oregon coast are in general from 9°-12°C, some growth advantage could be obtained through the use of heated seawater for culturing chum salmon. This is particularly true if an open coastal site is considered, because such a site would probably experience less of a problem with vibriosis and would have a lower ambient temperature than most estuarine locations.

In any case care would have to be used to see that culture temperatures did not exceed 14°C. Unless significant advances are made in the effectiveness of the vaccination procedures for salmon, it is unlikely that chum salmon can be commercially cultured at temperatures that consistantly exceed 14°C. This is because as long as *Vibrio* is a threat, the fish simply cannot tolerate any kind of stress, least of all a stress such as high temperature that concurrently favors the growth of *Vibrio*.

Our limited studies with pink salmon suggest that they may be faster growing than chum salmon, and that they may be able to tolerate somewhat higher temperatures than chum salmon. Good growth in very

young pink salmon occurred at temperatures up to 18°C. Larger pink salmon (greater than 50 g wet weight) grew best at about 14°C. In the absence of vibriosis, pink salmon may be more amenable to culture in heated seawater than chum salmon.