AN ABSTRACT OF THE THESIS OF

Maria Elena Diaz for the degree of Master of Science in Fisheries & Wildlife presented on March 3, 1992.

Title: Growth of Juvenile Pacific Oysters, Crassostrea gigas (Thunberg) and Manila Clams, Tapes japonica (Deshayes) in Effluent from Salmon-Macroalga Polyculture System.

Abstract approved:

William J. McNeil

Experiments were carried out in fall and winter, 1990 and spring and summer, 1991, to determine growth and mortality of juvenile Pacific oysters (Crassostrea gigas) in effluent from cultured coho salmon (Oncorhynchus kisutch), and effluent from salmon in which the red macroalga Palmaria mollis was cultured. Ambient sea water from Yaquina Bay was used as a control. Juvenile Manila clams (Tapes japonica) were also tested in summer, to compare the growth response of clams with that of Pacific oysters. Measured growth parameters included: increase in mean individual live weight, specific growth rate (% increase of mean live weight per day) and mean individual organic (ash-free) weight. Temperature, chlorophyll a,

phaeopigment, carbon and nitrogen concentrations and C/N ratio for all treatments were also recorded during spring and summer, 1991.

The oysters grew significantly faster in effluent from salmon and salmon conditioned by macroalgae than in the control during the Fall Experiment (September 7-October 31, 1990). Mean water temperature was 13-16°C. Growth rates were significantly greater in oysters cultured in salmon effluent than in the control during the Winter Experiment (December 7, 1990-February 15, 1991). However, growth was very poor due to low water temperatures (7-10°C).

Growth of oysters was significantly greater in the control than in effluent from salmon and salmon conditioned by macrolagae in the Spring Experiment (March 7-May 24, 1991). Mean water temperature was 12°C. In the Summer Experiments, (June 3-July 4 and July 19-August 17, 1991) growth of oysters was more rapid in treatments with macroalgae as compared to treatments without macroalgae Comparative experiments with juvenile Manila clams gave similar results.

Percentage mortality for both oysters and clams ranged from 0 to 5% and was highest during winter and lowest during summer. Mean chlorophyll values ranged from 1 μg/l in spring, 1991 to 11 μg/l summer, 1991.

Growth of Juvenile Pacific Oysters,

Crassostrea gigas (Thunberg) and Manila Clams,

Tapes japonica (Deshayes) in Effluent from

Salmon-Macroalga Polyculture System

by

Maria Elena Diaz

A THESIS

submitted to

Oregon State University

in partial fulfillment of

the requirements for the

degree of

Master of Science

Completed March 3, 1992

Commencement June 1992

APPROVED:

Professor of Fisheries and Wildlife in charge of major

Head of department of Fisheries and Wildlife

Dean of Graduate School

Date thesis is presented   March 3, 1992

Typed by Researcher

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF FIGURES

LIST OF FIGURES

LIST OF TABLES

Growth of Juvenile Pacific Oysters <u>Crassostrea</u> <u>gigas</u>
(Thunberg), and Manila Clams <u>Tapes</u> <u>japonica</u> (Deshayes)
in Effluent from Salmon-Macroalga Polyculture System.

INTRODUCTION

Aquaculture is the farming of marine organisms,
including fish, mollusks, crustaceans and aquatic plants.
The history of aquaculture dates back several centuries to
China, when carp were raised in rice paddies as a
complement to agriculture. Recent research has provided
the tools for commercial-scale farming of high-value
species under controlled conditions. Growth of catches
from capture fisheries has slowed even though the demand
for high-value aquatic foods continues to grow (McNeil,
1988). Fish farming has secured a sizeable niche in the
market for fish and shellfish, with considerable future
potential for development (Hepher and Pruginin, 1981).

Advances in aquaculture technologies were modest
before World War II. However, more recent advances in
technology have revolutionized operations with salmonid
fishes, crustaceans, and other aquatic resources (Lindsay
and Needham, 1988). The development of aquaculture has
created economic growth notably in coastal areas. In some
countries, it has made a positive contribution to regional
economies where few other alternatives exist (Huet, 1986).
In fact, in many countries aquaculture is presently the
only growth sector within fisheries. Over the last five
years, aquaculture enjoyed, on average, an annual growth

years, aquaculture enjoyed, on average, an annual growth rate of 4.25% worldwide (OECD, 1989).

A brief review of technical aspects of aquaculture has shown that aquaculture systems are diverse, employing a wide range of species and technologies. Systems develop gradually as an understanding of the biological requirements of candidate species for aquaculture improves (Quast et al., 1988). Examples provided by several European countries show that the efficiency of technologies can be substantially improved by appropriate refinement of operational procedures and by optimizing the design of system components (Huet, 1986).

Aquaculture systems have the potential to release nutrients and other wastes into the natural environment (Foy and Rossel, 1991). Pollutants from aquaculture operations have, in many cases, already become a risk factor for the industry (OECD, 1989). It is important for the aquaculture industry to maintain high quality of influent and effluent water. There are a number of possibilities to avoid negative impacts of pollutants. These include development of advanced technologies to reduce polluting loads derived from aquaculture, selection of sites which facilitate management of pollutants, and inclusion of additional aquaculture crops that utilize wastes from effluent waters before they are discharged (Chew, 1981; Else, 1987; Hargis and Haven, 1988). Fish,

for example, produce waste products which can be either utilized as food by filter-feeding bivalves or by plants in photosynthesis (Hartman et al., 1973, Jones and Iwama, 1991). Substances in the water and sediments of an aquaculture system include mineral salts and various carbon, nitrogen, and phosphorous compounds that can be successfully utilized both by phytoplankton and macroalgae in photosynthesis (Mann and Ryther, 1977). Even though phytoplankton are generally considered to be the main source of food for mollusks and other filter-feeding animals, it is now known that benthic plants (i.e. macroalgae) also appear to be important contributors to food webs involving suspension feeders in estuarine and coral reef habitats (Newell and Lucas, 1981; Newell and Field, 1983). Mann (1972; 1973) and Duggins (1989) showed exceptionally high productivity of benthic macrophytes belonging to the order Laminariales (kelp) and inferred that kelp-derived organic carbon could play a significant role in temperate coastal (nearshore) secondary production. By releasing particulate as well as dissolved organic matter, kelp appears to provide a significant organic carbon source for the diverse and abundant assemblages of nearshore pelagic and benthic suspension feeders (Newell and Lucas, 1981). Many heterotrophic organisms have been shown capable of carbon fixation (Hammen and Wilbur, 1959; Hammen, 1966). Lamellibranchs

are capable of absorbing dissolved organic matter from their surrounding environment (Stephens, 1967; Wright, 1982; Rice and Stephens, 1987; Wright, 1988). Oysters, for example, can remove amino acids from seawater (Manahan and Crisp, 1982; Manahan, 1989). Crosby at al. (1990) found evidence that cellulolytic bacteria in the environment can contribute to the transfer of carbon from refractory detritus to oysters (Crassostrea virginica). Their calculations show that detrital complexes in the natural environment may provide a significant contribution to an oyster's carbon demand. Duggins et al. (1989) demonstrated that growth rates of benthic suspension feeders were greatly increased in the presence of organic detritus originating from benthic macroalgae. Moreover, stable carbon isotope analyses confirm that kelp-derived carbon is found throughout the nearshore food web (Newell and Field, 1983).

Cultured mollusks account for more than 85 % of global shellfish aquacultural production (O.C.D.E., 1989). The three species of mollusks that make up the majority of global aquacultural production are oysters, sea mussels and clams (Epifanio et al., 1975). The oyster is perhaps the most prominent among the various marine species presently considered appropriate for intensive and controlled culture.

Ryther and Bardach (1968) have described certain

biological criteria that should be satisfied in order for a species to be adaptable to culture: responsiveness to efforts to effect reproduction under captive conditions; tolerance of eggs and larvae to the culture environment; nutritional requirements that can be satisfied in this environment, and a relatively rapid rate of growth from egg to maturity. To a large extent, the oyster satisfies these criteria, and it possesses other favorable qualities as well. The oyster is highly fecund, a single female being capable of producing many millions of eggs in a single spawning (Loosanoff and Davis 1963). Since it is omnivorous, the oyster's nutritional requirements can be more readily satisfied than are those of a carnivore. Finally, and of considerable importance with respect to economic considerations of aquaculture, oyster culture is potentially highly profitable and is a traditional industry in the majority of coastal states (Angell, 1986; Boyle, 1989).

The North American oyster industry relies largely upon the production of two species: _Crassostrea virginica_ and _C. gigas_, the former being the primary species cultured on the East Coast and Gulf States U.S.A., while _Crassostrea gigas_ is the most important cultured oyster species on the Pacific Coast (Matthiessen, 1971). _C. gigas_ (Thunberg) is the oyster known as the "Miyagi" in Japan, as the Pacific oyster in the United States, and as

the Japanese oyster in Europe. It was first introduced to North America as the Miyagi strain imported from Japan at the turn of the century. Up to the 1970's, seed was annually brought from Japan because the waters of the Pacific Northwest were too cold to allow complete gametogenesis and spawning (Boyle, 1989).

Development of a hatchery technology for artificial production of seed oysters began in the 1940's and 1950's. However, it was not until the 1970's that it became widely adopted by the West coast oyster industry.

With the introduction of Crassostrea gigas, the west coast oyster industry has experienced a considerable change. This species is extremely hardy and its growth surpasses that of all other cultivated oysters.

Growth in C. gigas can be extremely variable, and is dependent upon a number of factors, including location in the water column, water temperature, salinity, current velocity, phytoplankton and/or particulate matter concentration (Walne, 1972; Walne and Spencer, 1974). Pacific oysters tolerate water temperatures ranging from freezing to more than 30°C, but feeding will cease at temperatures less than 5°C. Optimum water temperatures for maximum growth rate are said to be about 15°C (Bardach et al., 1972; Malouf and Breese 1977, 1978). Salinities tolerated by Pacific oysters the range from 5 to 37 ppt. The optimal salinity range is 10 to 30 ppt (Quayle, 1969).

Oysters can live in a poorly oxygenated environment (about 2 ml/O_2/l) for several days with their valves closed, and this ability increases at lower temperatures (Kafuku and Ikenoue, 1983).

The Pacific oyster is able to tolerate turbidity. However, extremely turbid water may reduce feeding rates (Quayle, 1969; Shumway et al., 1985). Small amounts of silt or sediment can stimulate feeding and growth (Loosanoff, 1962; Urban and Langdon, 1984).

The rate at which oysters filter water depends mainly upon water temperature and the quality of the food in the water (Quayle, 1969; Walne, 1972; Malouf and Breese, 1977). Adult Crassostrea virginica filters several gallons of seawater per hour at 20°C and 25-35 ppt (Glaude, 1977), and ingests food particles within a 10-100 μ size range (Walne, 1972, Chew, 1981).

Malouf and Breese (1978) found that the optimum temperature for growth of juvenile Pacific oysters was about 15°C. High temperatures (above 20°C) resulted in reduced growth and high mortality. In addition, they reported extreme seasonal fluctuations in the growth of oysters under constant conditions of water flow and temperature, with little or no growth recorded between October and March. In another series of experiments, they showed that there is no single relationship between water flow and oyster growth at different temperatures, because

the water flow requirements are ultimately determined by the food content of the water.

The Manila clam is one of the species that has lately attracted the attention of aquaculturists because of its high market demand. It was accidentally introduced to the west coast of North America with Pacific oysters imported from Japan (Chew, 1989). They have become the second most important commercial clam species on the West coast after the geoduck, Panope generosa, in terms of pounds landed and economic value. There is great disparity among reported growth rates and sizes of juvenile Manila clams from different locations (Chew, 1989; Willjams, 1980b) as well as for temperatures associated with optimum growth (Mann, 1979; Anderson, 1982). Reported optimum temperatures for growth of Manila clams range from as low as 12°C (Langton et al, 1977) to as high as 23-24°C (Bardach et al., 1972; Langton et al., 1977; Glock and Chew, 1979; Bourne, 1982; Goulletquer et al., 1988). High mortalities of the clam during winter and summer have been associated with extreme substrate or water temperatures (Chew, 1989).

Culture of Manila clams (Tapes japonica) and Pacific oysters in the same aquaculture system may hold potential for future commercial applications because these two species appear well suited for joint culture. Since they naturally grow under similar intertidal conditions, their

environmental requirements for growth might be nearly the same (Bourne, 1989). The advantage of growing bivalves as secondary crops in pump-ashore mariculture systems is that the two main causes of bivalve mortality in the natural environment, extreme siltation and predation, can be greatly reduced or eliminated (Bayes 1981; Williams, 1981). Also, polyculture is economically attractive because food costs could be significantly reduced by utilizing the nutrients provided by aquacultural waste effluent to feed bivalves. Commercial nursery culture of bivalve mollusks depends on natural rather than artificial foods (Spencer et al., 1986). Hatchery costs per oyster fed on cultured microalgae can represent up to 17% of its commercial value.

A good example of the high production efficiency of polyculture systems was reported by Ryther et al. (1974). They utilized domestic waste water effluent from a secondary sewage treatment, mixed with seawater, as a source of nutrients for growing unicellular marine algae. The algae, in turn, could be used as food for oysters, clams and other bivalve mollusks. Solid wastes from the shellfish were consumed by polychaete worms, amphipods, and other small invertebrates that, in turn can be used as food for lobsters or other commercially valuable secondary crops. In their system, Ryther et al. (1975) utilized several species of macroalgae (<u>Chondrus</u> sp, <u>Gracillaria</u>

sp, _Agardhiella_ sp) to remove dissolved waste products excreted by shellfish and other crops as a final "polishing" step.

The research presented here integrates existing technologies for growing fish (salmon), macroalgae, and bivalve mollusks into a polyculture system. This research was undertaken to evaluate the inclusion of Pacific oysters into the system and to compare growth of Pacific oyster with that of Manila clams under the same culture conditions. The polyculture system used nutrient-enriched waste effluent from coho salmon (_Oncorhynchus kisutch_) to produce a crop of macroalgae (_Palmaria mollis_). Strategies are being explored to incorporate bivalve mollusks (specifically oysters and clams) in effluent water from either salmon and macroalgae and on the salmon effluent alone.

The overall objective of this research was to determine the effect of effluent water from cultures of salmon and red macroalgae, _P. mollis_, on the growth of juvenile Pacific oyster. The specific objectives of this research were:

1. To evaluate growth of juvenile Pacific oysters raised in effluent from cultured salmon and effluent from cultured salmon conditioned by _P. mollis_.

2. To evaluate the effect of effluent from cultured salmon conditioned by different stocking densities of P. mollis on the growth of juvenile Pacific oysters.

3. To compare relative growth of juvenile Pacific oysters and juvenile Manila clams in effluent from salmon and salmon conditioned by P. mollis.

MATERIALS AND METHODS

Experiments were carried out at the Hatfield Marine Science Center, Oregon State University, Newport, Oregon. Juvenile oysters, weighing 40 to 90 mg were used in all the experiments. Cultchless (unattached to substrate) juvenile oysters were obtained from Kuiper Mariculture Eureka, California. They were sorted according to size by sieving to obtain test animals of uniform size. Two groups of juvenile Manila clams, having average individual weights of 15 and 63 mg, respectively, were used in Summer Experiments # 1 and # 2, to compare clam growth rates with those of oysters. Clams were also obtained from Kuiper Mariculture. Groups were randomized among treatments for each experiment.

Ambient seawater, pumped from Yaquina Bay was stored in a settlement tank before experimental use. Ambient seawater was either directly utilized for bivalve culture or conditioned by exposure to fish (salmon) and macroalgae. Salmon effluent water was obtained from a 12,900 l tank stocked with juvenile coho salmon at a commercial hatchery density of 9 kg/m^3. Salmon weighed in average 125 g and were fed a daily maintenance ration at 2% of their body weight. Salmon were fed on a commercial salmon diet (Silver Cup, Murray Elevators) using an automatic feeder. The salmon culture tank was covered

with black mesh netting to reduce light and fouling by epiphytes.

Experiments were performed over a period from September 7, 1990, to August 17, 1991. Effluent water from salmon was delivered, in some instances, to experimental units containing test groups of bivalve mollusks without exposure to red macroalgae and in other instances after exposure to red macroalgae. The methodology for exposing water to red macroalgae varied among experiments as described in this thesis.

Experimental Treatments

There were five principal experiments, conducted at different seasons of the year (two experiments were conducted during summer, 1991). Each experiment is discussed separately under subheadings of "Fall", "Winter", "Spring" and "Summer" experiments. The "Summer" experiments will be referred to as "Summer Experiment #1" (June 3 - July 4, 1991) and "Summer Experiment #2" (July 19 - Aug 17, 1991), both of which compared the growth of Pacific oysters with that of Manila clams in the same culture system. A shorthand terminology for treatments used in experiments will be introduced in the Methodology section to facilitate further presentation of results and discussion.

<u>Fall Experiment</u>

This experiment was conducted during a 7 week period (from September 7 through October 31, 1991). Its primary purpose was to test the effect of the following treatments on growth of juvenile Pacific oysters:

1. Salmon effluent conditioned by the addition of 10.4 g/l <u>Palmaria mollis</u> (F+A).

2. Salmon effluent alone (F-A)

3. Ambient seawater conditioned by the addition of 10.4 g/l <u>P. mollis</u> (R+A).

4. Ambient seawater alone (R-A)

A gravity flow upweller system was used for this experiment. This culturing technique allows water to flow upwards to bring food to the oysters and to remove feces and organic wastes (Spencer and Hepper, 1981; Manzi et al. 1986). Two sources of water, salmon effluent and ambient seawater, were pumped separately to two 400 l head tanks, elevated 3 m above the ground. Water was first delivered to 20 polyethylene containers (volume = 11 liters, Fig. 1), before being distributed to individual upwellers made of 12" lengths of gray PVC pipe (2 1/2" of diameter, Fig. 2). The polyethylene cells were stocked with either 0 or 10.4 g/l (live weight) of <u>P. mollis</u>. The stocking density for the alga was based on seasonal light availability, according to data reported by Levin (1990).

Groups of experimental animals were randomized among
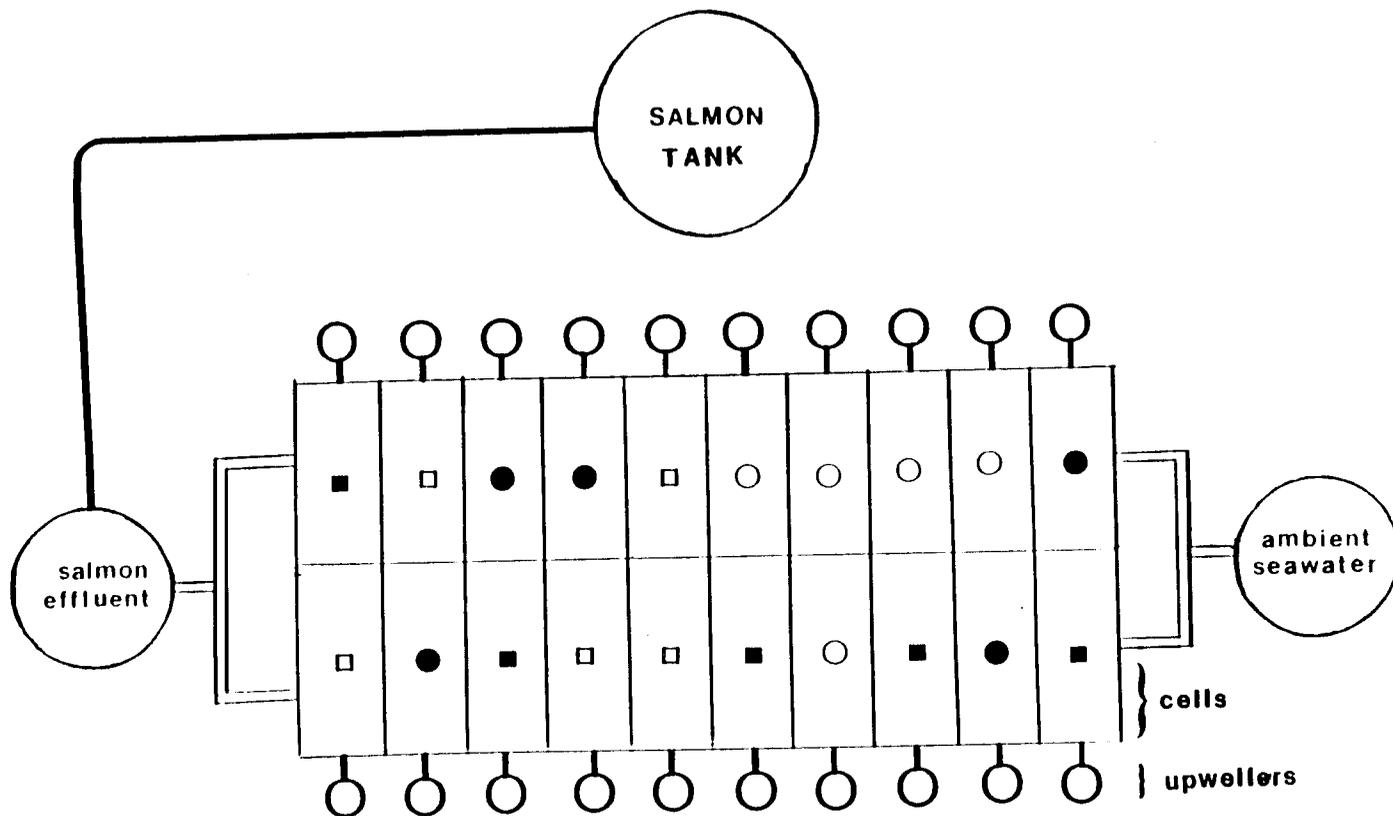
FIGURE 1.- Experimental design, Fall Experiment (Sep 7 - Oct 31, 1990). Each cell delivered water from different treatments to individual upwellers stocked with 10 g each of juvenile Pacific oysters. Symbols represent: (■)= salmon effluent conditioned by 10.4 g/l of _Palmaria mollis_; (□) salmon effluent alone; (●)= ambient seawater conditioned by 10.4 g/l of _P. mollis_; (O)= ambient seawater alone.
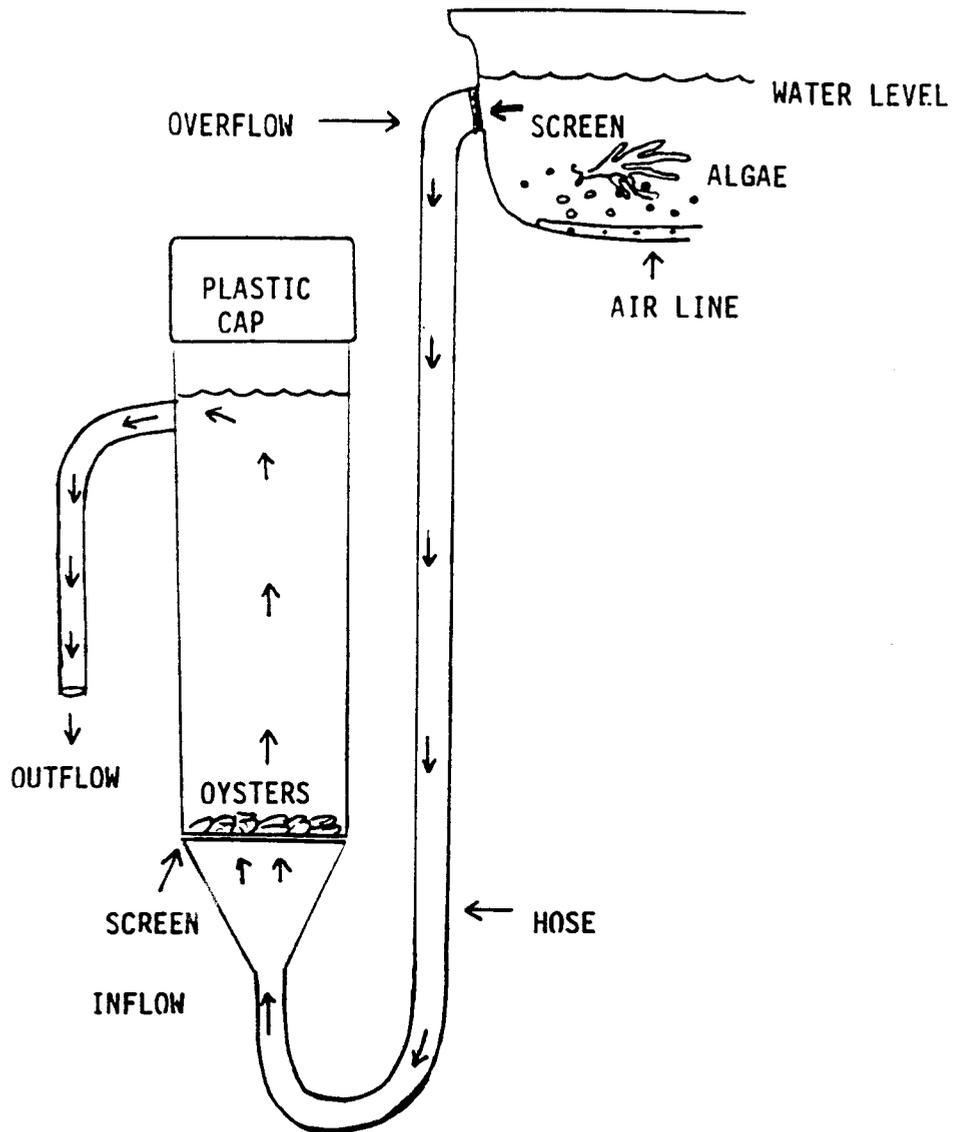
FIGURE 2.- Detail of a polyethylene container stocked with <u>Palmaria</u> <u>mollis</u> and gravity flow upweller utilized to grow juvenile Pacific oysters during Fall Experiment (Sep 7 - Oct 31, 1990). Initial stocking density of oysters was 10 g per upweller.

five replicates for each treatment. Each upweller was stocked with 10 g (live weight) of juvenile oysters (between 119 - 136 animals per tube). The mean initial live weight of each animal was 78 mg. During a previous experiment, performed under controlled laboratory conditions, filtration rates were determined for groups of 10 juvenile oysters of similar sizes to those of oysters used in the growth experiments. Each oyster filtered an average of 40 ml/h seawater enriched with 30,000 cells/ml of Isochrysis galbana at 16°C. This information, along with filtration rates reported from the literature (Jorgensen, 1966; Malouf and Breese, 1978; Rodhouse and O'Kelly, 1981) for juvenile Pacific oysters, determined the minimum flow rates to be used in this experiments so that oysters would have an adequate food supply. For the Fall Experiment, water flow to the polyethylene containers was regulated by means of individual valves which were set and maintained at 35 ml/min/g of oyster.

The daily maintenance routine for the system included checking and maintaining flow rates, scrubbing the macroalga containers to eliminate fouling by epiphytes and encrusting organisms, and hosing the inside of the upwellers and water supply lines to maintain flow rates. Head tanks were cleaned once a week. The alga was removed from the polyethylene cells, rinsed, centrifuged, weighed and restocked at the same prescribed density once a week.

Wet weight of test oysters was determined at the end of 2, 5 and 7 weeks. Water temperature was recorded daily in the afternoon.

Winter Experiment

This experiment started on December 7 and ended on February 15. The main objective was to test the effect of salmon effluent and two different stocking densities of $\underline{P}$. $\underline{mollis}$ on the growth of juvenile oysters.

The treatments tested were:

a) Effluent from cultured salmon conditioned by addition of 7 g/l $\underline{P}$. $\underline{mollis}$ (F+A).

b) Effluent from cultured salmon conditioned by addition of 12 g/l $\underline{P}$. $\underline{mollis}$ (F+A2).

c) Effluent from cultured salmon alone (F-A).

d) Ambient seawater alone (R-A).

Airlift upwellers were used for this experiment (Fig. 3). The actual configuration and dimensions of these are the same as the ones used in the previous experiment (12" lengths of gray PVC pipe, 2 1/2" in diameter) but here the flow of water was maintained by an air-lift system instead of gravity. Four fiberglass tanks (104 l of capacity each) received water from the experimental sources (salmon effluent and ambient seawater). Eight airlift upwellers containing test groups of oysters were immersed in each tank. Average size, weight and initial stocking density

OUTFLOW

AIR
BUBBLES

OYSTERS

INFLOW

AEREATOR

SCREEN

ALGAE

AIR PIPE
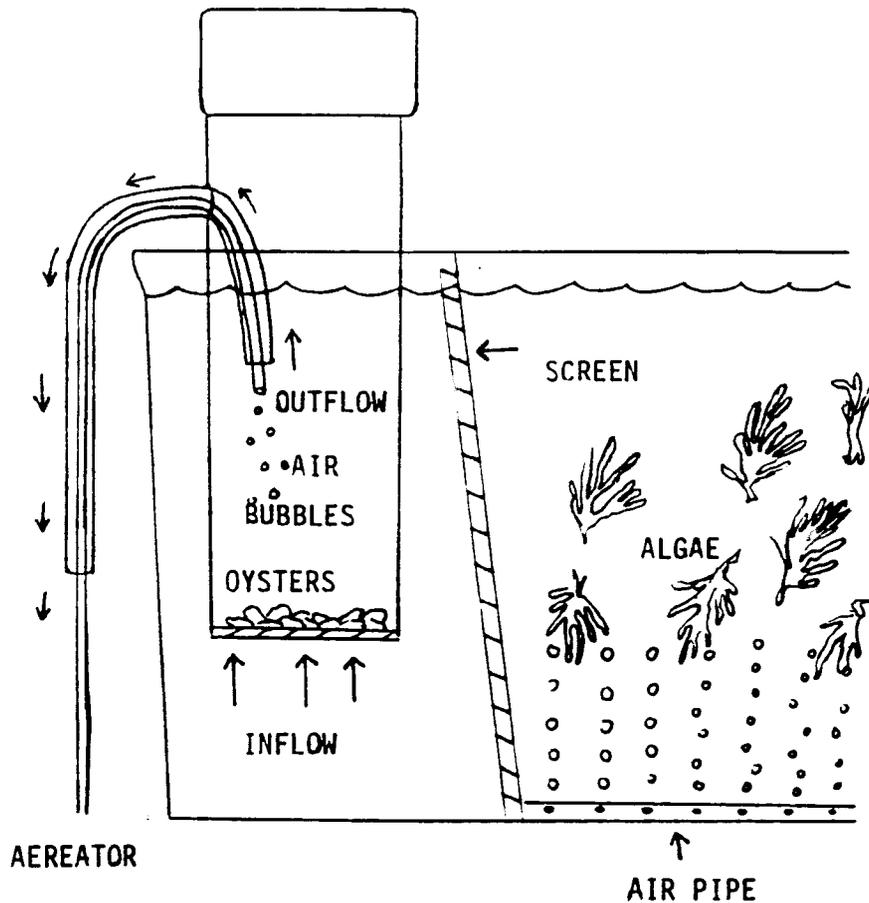
FIGURE 3.- Experimental apparatus, Winter Experiment (December 7, 1990 - February 15, 1991). Salmon effluent conditioned by 7 and 12 g/l _Palmaria_ _mollis_ was delivered to airlift upwellers initially stocked with 10 g/each of juvenile Pacific oysters.

of the oysters was the same as for the Fall Experiment. Effluent water from tank-cultured salmon and ambient seawater were delivered into the four tanks at a flow rate of 2.7 l/min. Water in excess of the amount delivered through the upwellers was discarded trough an overflow drain. Temperature was recorded every day. Routine maintenance of the system was the same as that described for the Fall Experiment.

Spring Experiment

This experiment ran from March 7 through May 24. The purpose of this experiment was to test the effect of the following treatments on the growth of juvenile Pacific oysters during spring:

1. Salmon effluent conditioned by addition of 40 g/l P. mollis (F+A2).

2. A mixture of half the effluent in treatment #1 (F+A2) and half ambient seawater (50%-50%). This treatment was (F+A).

3. Salmon effluent alone (F-A).

4. Ambient seawater alone (R-A).

Treatment #1 had twice the stocking density of macroalga that results on maximum macroalgal yield in March based on light availability according to Levin (1990). In treatment #2, ambient seawater was added to dilute the nutrients in treatment #1 to half its strength

(50%-50%). The culture apparatus used in this experiment was different from that described for pervious experiments. Test animals were placed in small, rectangular plastic containers (450 ml volume) which received water by gravity flow from four 75 l reservoirs supplied with either ambient seawater or salmon effluent with or without conditioning by addition of P. mollis (Fig. 4-5).

Five grams of juvenile oysters with average individual weight of 90 mg were stocked in each one of the boxes (50 to 53 oysters per container). Water was delivered to each experimental unit at a rate of 70 ml/min/g of oyster. The water reservoirs were cleaned every other day. The 450 ml plastic containers and the oysters were flushed and rinsed daily with ambient sea water to remove debris and other waste material. The water reservoirs and experimental units were covered to avoid fouling by epiphytic organisms. The tank containing the alga was cleaned once a week, and the amount of alga was adjusted to its initial density. Chlorophyll a and phaeopigment concentrations (μg/l) were determined by fluorometry (Strickland and Parsons, 1984) for each treatment on May 13, 20 and 24.

FIGURE 4.- Detail of the experimental units (n=6) utilized to grow juvenile Pacific oysters during Spring Experiment (Mar 7 - May 24, 1991). Each unit was initially stocked with 5 g of oysters.
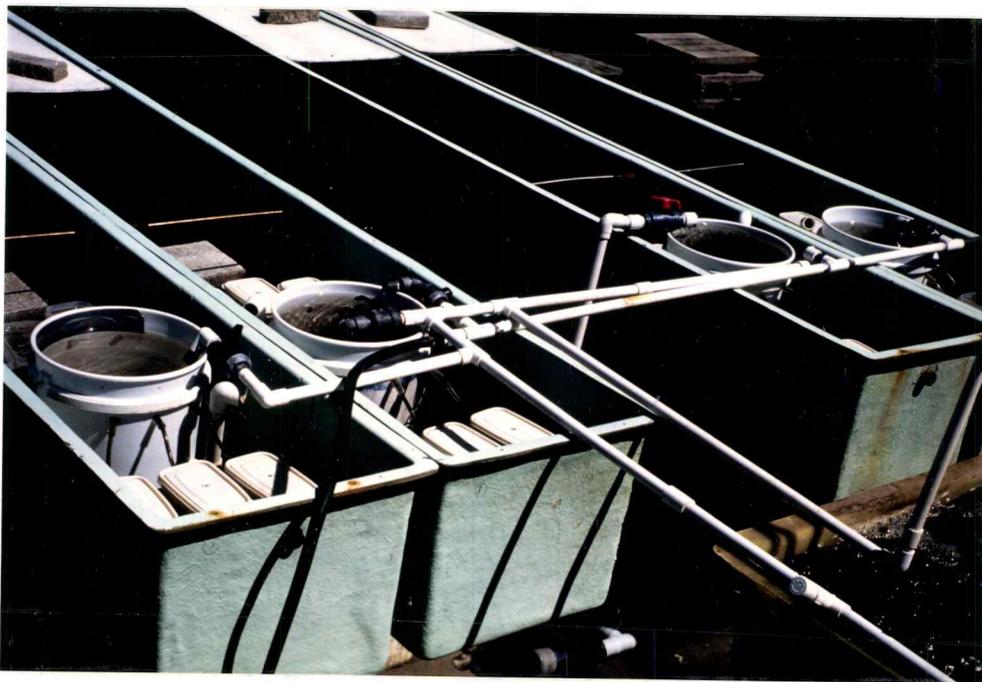
FIGURE 5.- Experimental apparatus, Spring Experiment (March 7 - May 24, 1991). The water reservoirs and the experimental units were kept in raceways and then covered.

Summer Experiments

Two similar experiments were performed during summer, 1991: the first one was carried out from June 3 through July 4, 1991 and the second one from July 19 through August 17, 1991. The culture apparatus used for the Summer Experiments was similar to that described for the Spring Experiment. Treatments tested were the same as for the Fall Experiment, but algae were stocked at 18 g/l in the Summer Experiments. Tested treatments were:

1. Salmon effluent water conditioned by the addition of 18 g/l macroalga (F+A).

2. Salmon effluent water alone (F-A).

3. Ambient seawater conditioned by the addition of 18 g/l macroalga (R+A).

4. Ambient seawater alone (R-A).

Algae were grown in two 104 l fiberglass tanks stocked with 18 g/l P. mollis, each one receiving salmon effluent or ambient sea water from the main water sources (Fig. 6-7). Groups of juvenile Manila clams (average individual live weight of 15 mg) and Pacific oysters (mean individual live weight of 60 mg) were used as test animals. The experimental units holding them were randomized so that three of them were each stocked with 5 g of clams and the other three with 5 g of oysters for

FIGURE 6.- Experimental apparatus, Summer Experiments. Initial stocking density of bivalves was 5 g per replicate (n=3) during Summer Experiment #1 (June 3 - July 4, 1991) and 3 g in Summer Experiment #2 (July 19 - Aug 17, 1991).

FIGURE 7.- Experimental apparatus, Summer Experiments. Upper tanks: salmon effluent (left) and ambient seawater (right) stocked with 18 g/l Palmaria mollis. Lower tanks hold the water reservoirs and the experimental units.

Summer Experiment #1. Stocking densities of animals were reduced to 3 g per replicate during Summer Experiment #2. Average initial weight per oyster and clam during Summer Experiment #2 were 47 and 62 mg respectively. Cleaning routine included daily flushing and rinsing of animals and experimental units. Water flow rates were 70 ml/min/g of bivalves during Summer Experiment #1, and 117 ml/min/g of bivalves for Summer Experiment #2. Bivalve biomass was reduced in the second experiment of summer to ensure maximum food availability for the animals in the experimental units. The alga was weighed and stocking density was adjusted to its initial level every week, as in previous experiments.

Growth Determinations

Live weights of test groups were determined at the beginning of each experiment and every 2 weeks during Fall, Spring, and Summer Experiments, and every 3 weeks during the Winter Experiment. At each weighing, the bivalves were taken out of the experimental units and rinsed with ambient seawater, blotted with a paper towel and allowed to air-dry for 30 minutes. Oysters were counted and mortalities were removed and recorded. The number of surviving oysters per replicate per treatment was also recorded. A Mettler PC 4400 digital scale was

used to determine the total combined weight of the bivalves in each replication to the nearest 10 mg. After weighing, the bivalves were returned to the experimental units. When an experiment was terminated, the test animals were oven-dried at 60°C for 48 hrs, then baked in a furnace at 450°C for another 48 hours. Dry and organic weights were recorded for each test group on a Mettler AC 100 digital scale to the nearest 0.1 mg. The total organic weight per test group was divided by the number of survivors to obtain an estimate of the average organic weight per animal.

Specific Growth Rate (percentage of increase in live weight per day) was calculated for the test groups in each replicate after each weighing according to the following formula (Ricker, 1975):

$$SGR = \frac{\ln [\ W_t/n_t\ /\ W_o/n_o\ ]}{t} \times 100$$

Where:

    W_t = Weight of each test group at time t.

    n_t = Number of animals at time t.

    W_o = Weight of each test group at time 0.

    n_o = Number of animals at time 0.

    t = time of experimental period in days

Water Quality Analyses

On June 13, 1991, samples of 40 l of water from each of the four treatments were taken, centrifuged on a Sorvall RC-2 centrifuge at 12,000 rpm at a flow rate of 100 ml/min and freeze-dried on a Virtis Freezemobile 6 Lyophilizer for 48 hrs. The weight of the suspended particulate matter obtained in this way was recorded and divided into several subsamples to perform the following analyses:

Chlorophyll determinations

Chlorophyll a and phaeopigment determinations were carried out by fluorometry, following the method described by Strickland and Parsons (1984). Two replicates of approximately 10 g of sediment from each water treatment filtrate were placed in 10 ml, baked disposable glass tubes; 5 ml of 90% acetone and 2 drops of magnesium carbonate were added and each tube was sealed with teflon tape and sonicated in an ultrasonic bath for 2 min and frozen overnight. The next day, after thawing and allowing to warm to room temperature, the tubes were centrifuged at 4,000 rpm for 5 min. Chlorophyll a and phaeopigment contents were determined by measuring fluorescence of the samples before and after acidification with 1 N hydrochloric acid in a Turner fluorometer; 90%

acetone was used to dilute the samples when needed.

The methodology to determine chlorophyll a and phaeopigment in spring and in the Summer Experiment #2 involved the analysis of 100 ml water samples. Three replicates were collected from each one of the treatments. The water samples were vacuum filtered through GF/F filters and the filter added to 10 ml of 90 % acetone, and 0.5 ml of magnesium carbonate. The rest of the procedure was the same as described for determining chlorophyll concentrations of samples of suspended particulate matter. Water samples for chlorophyll determinations were taken on May 13, 20 and 24, 1991, for Spring experiment, and on July 6 and August 13, 1991, for Summer Experiments #1 and #2.

Total Organic Contents

Two samples of 20 mg of freeze-dried suspended particulate matter from each water treatment were transferred to baked aluminum foil cups and weighed on a microbalance. The cups were placed in numbered crucibles and baked at 500°C for 12 hours. Crucibles were then transferred to mini desiccators and allowed to cool. Loss of weight of the sample due to loss of organic matter was recorded on a digital scale to the nearest 0.1 mg.

C/H/N Analysis

Two samples of 20 mg of sediment from each water treatment were sent to the Marine Science Institute Analytical Laboratory at the University of Santa Barbara California, to determine content of carbon, hydrogen, nitrogen, and carbon/nitrogen ratio (Levin, 1990).

Statistical Analyses

All statistical analyses were performed with the computer programs Statistical Analyses System (S.A.S.) version 6.04 and Statgraphics, version 4.0. A normal probability plot was used to test for normality of the data and Levene's test (Snedecor and Cochran, 1980), as well as graphical displays of residuals versus predicted values (Schaffer, 1989) were used to test for homogeneity of variances. Some of the data were transformed to logarithmic values in order to meet the assumptions of ANOVA. For Fall and Summer Experiments, a two-way ANOVA with two factors (fish and alga) was utilized to determine statistically significant differences in weight increases for treatments at the end of the experimental period. A one-way ANOVA was utilized to analyze data from the Spring Experiment. Where significant differences were indicated, Tukey's Honestly Significant Difference test (T-HSD) was applied to determine the statistical significance of

differences among individual treatments at the 0.05 level of significance. Specific growth rates data from the last measurement in the Winter Experiment were analyzed by a non-parametric procedure, because of heteroscedasticity of variances that could not be removed by transformations. A Kruskall-Wallis test (Sokal and Rohlf, 1969) was used in lieu of single classification ANOVA and a Wilcoxon test (Sokal and Rohlf, 1969) was used as a posteriori method to determine significant differences among means. All statistical tests were performed at the 0.05 level of significance.

RESULTS

Results are presented under two major subject
headings:

1. Monitoring of Environmental Variables

2. Production of Bivalves

Monitoring of Environmental Variables

This section deals with results on temperature,
chlorophyll a and phaeopigment levels and other selected
water parameters (C, H, N and C/N ratios) determined
throughout the experimental period.

Water Quality

Water quality parameters determined from the
suspended particulate organic matter samples taken from
each one of the treatments on June 13 are shown on Table
1. Carbon, H and N concentrations were highest in the F+A
and F-A treatments. Carbon and H concentrations and C/N
ratio were highest in treatments without alga (F-A, R-A).
Chlorophyll concentrations were higher in F+A and F-A
treatments, averaging 2.5 μg/l. R-A treatment had the
lowest chlorophyll a concentration (0.88 μg/l).
Concentration of particulate organic matter were highest
in R-A treatment, compared to those of other treatments.

Table 1. Suspended particulate matter sample analyses (N=2) for Summer Experiment #1 (June 3 - July, 4 1991). Carbon (C), Hydrogen (H), Nitrogen (N), chlorophyll a (Chl-a) and phaeopigment (Phaeo) concentrations are expressed in μg/l. Carbon:Nitrogen ratio (C:N), Total Suspended Pariculate Matter (TSPM), and Particulate Organic Matter (POM) are also given. Treatments: F+A= salmon effluent and 18 g/l of Palmaria mollis; F-A= salmon effluent alone; R+A= ambient seawater and 18 g/l of P. mollis; R-A= ambient seawater alone (control).

| | μg/l | | | | | μg/l | | |
TREAT.	C	H	N	C/N	POM	Chl-a	Phaeo	TSPM
F+A	283	58	47	6.04	870	2.50	2.55	2,480
F-A	319	68	43	8.91	1,180	2.60	2.83	2,950
R+A	194	46	26	7.38	730	1.74	2.50	2,550
R-A	217	54	29	11.02	1,280	0.88	2.25	3,610

Temperature

   Temperature in the control (R-A treatment) varied throughout the experimental period from a minimum of 7°C in December, 1990, to a maximum of 17°C in September, 1990. Monthly mean water temperatures for the ambient seawater are shown in Fig.8 . Mean water temperature varied little between treatments throughout the year, except during summer. During that season, mean water temperature in the treatments conditioned by P. mollis was significantly higher (P<0.001) than the treatments without alga (Table 2). Water in these treatments was warmed as it passed through the macrolaga culture system, due to increased solar radiation because of longer and sunnier days in summer. A two way ANOVA with two factors, fish and alga, also revealed a highly significant effect (P<0.001) of the alga on higher mean water temperatures for F+A and R+A treatments. Results of the ANOVA table on those tests are presented in appendix 1.

   Upwelling episodes are usually correlated with high nutrient and phytoplankton concentrations (Broecker and Peng, 1982). A highly positive Bakun upwelling index was recorded (NOAA, NMFS, 1990-1991) for 45°N, 125°W (5 miles offshore Yaquina Bay) during spring and summer, 1991, (Fig. 9) suggesting that during the Spring and Summer Experiments the ambient seawater was richer in food than during Fall and Winter Experiments.
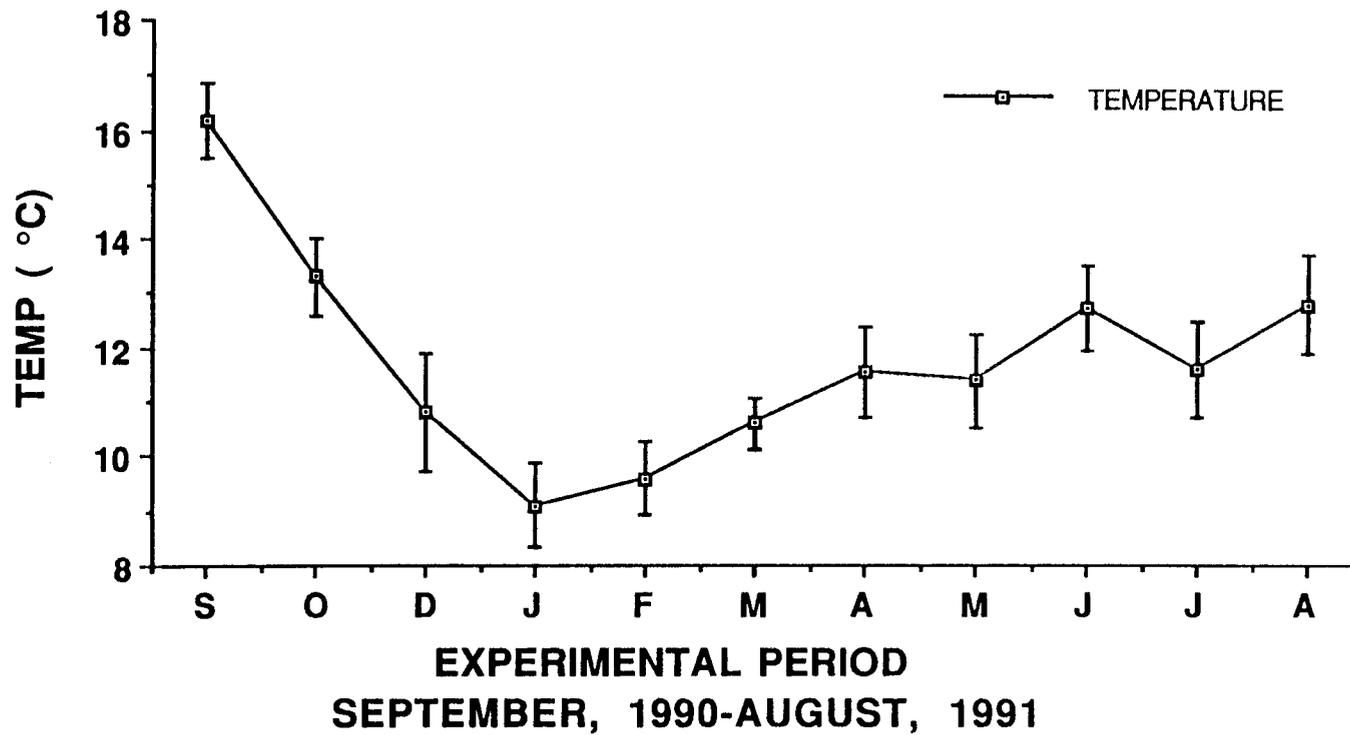
FIGURE 8.– Monthly mean water temperatures (°C) and standard errors of the means for the ambient seawater treatment (control), during the experimental period September 7, 1990 – August 19, 1991. No experiment was performed in November.

Table 2. Mean water temperatures and standard errors of the means (n=15) for treatments tested in Summer Experiment #1 (June 3-July 4, 1991) and Summer Experiment #2 (July 19-Aug 17, 1991). Legend: F+A= salmon effluent conditioned by 18 g/l _Palmaria_ _mollis_; F-A= salmon effluent alone; R+A= ambient seawater conditioned by 18 g/l of _P_. _mollis_ and R-A= ambient seawater alone (control). Means that differ statistically (P<0.001) as determined by Tukey's test are marked with an asterisk under "homogeneous groups".

Summer Experiment #1

TREATMENT	AVERAGE	S.E.	HOMOGENEOUS GROUPS
F+A	14.36	0.25	*
R+A	14.30	0.17	*
F-A	13.26	0.25	*
R-A	12.74	0.20	*

Summer Experiment #2

TREATMENT	AVERAGE	S.E.	HOMOGENOUS GROUPS
F+A	13.96	0.07	*
R+A	13.90	0.14	*
F-A	12.06	0.21	*
R-A	11.60	0.22	*
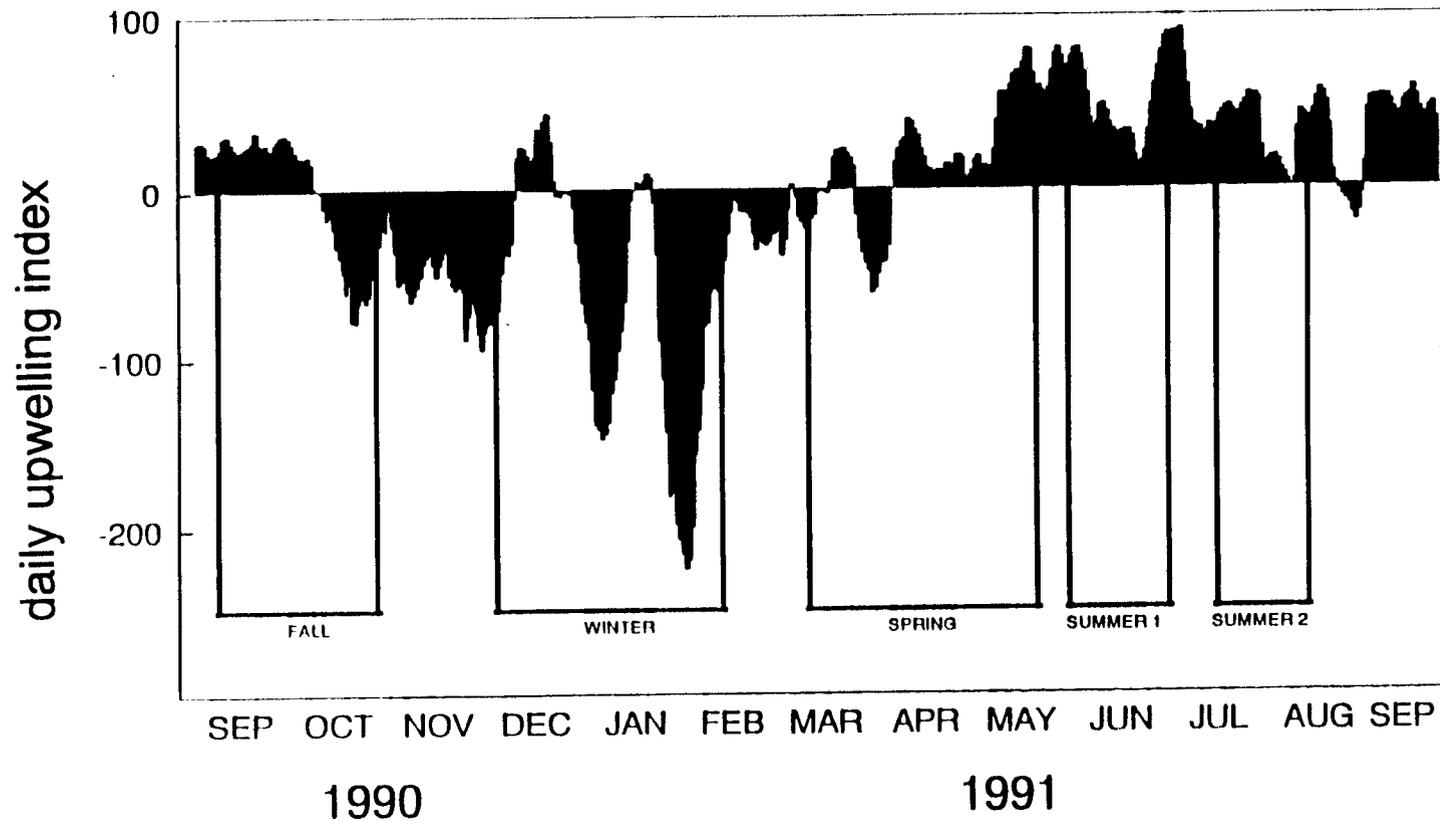
FIGURE 9.- Bakun daily upwelling index for 48° N, 125° W, 11 day
moving average during the experimental period (September 7, 1990 -
August 19, 1991). Source: NOAA, NMFS Database 1990 - 1991.

Chlorophyll Levels

Chlorophyll determinations were carried out for this study during Spring and Summer Experiments, 1991. Chlorophyll a concentrations varied little between treatments at each sampling date. Greatest differences among treatments were determined for concentrations of suspended particulate samples taken from each water treatment on June 13, 1991. Phaeopigment concentrations were always higher than chlorophyll a concentrations, and both increased as the spring turned into summer. Phaeopigment concentrations varied much more than chlorophyll a both among treatments and between sampling dates. Chlorophyll a concentrations varied from 0.92 to 1.57 μg/l during the spring and from 2.5 to 12.6 μg/l during summer (Tables 3-4), with little variation among treatments within a sampling date. Higher chlorophyll a variation among treatments were found late in summer.

Production of Bivalves

Growth of test oysters varied according to the season in which a particular experiment was performed (Fig. 10). There seemed to be a beneficial effect of the salmon or the salmon and macroalga effluents when water temperatures were still within a reasonable range for oyster growth. In fall, growth of oysters was significantly increased by

Table 3. Means and standard errors of chlorophyll a (Chl-a) and phaeopigment (Phaeo) concentrations (μg/l) from 100 ml water samples taken at various dates during the Spring Experiment (March 7 - May 24, 1991). Each value represents an average of three replicates. Treatments: F+A2= salmon effluent and 40 g/l of _Palmaria mollis_; F+A= a mixture of salmon effluent with 40 g/l of _P. mollis_ and ambient seawater (50%-50%); F-A= salmon effluent alone; R-A= ambient seawater alone (control).

SAMPLING DATE	F+A2		F+A		F-A		R-A	
	Chl-a	Phaeo	Chl-a	Phaeo	Chl-a	Phaeo	Chl-a	Phaeo
MAY 13	1.026	2.380	1.096	2.806	1.503	1.916	1.270	4.296
	(±.016)	(±.020)	(±.003)	(±.033)	(±.046)	(±.076)	(±.030)	(±.166)
MAY 20	1.007	2.363	1.074	2.863	1.052	3.630	0.992	3.836
	(±.014)	(±.028)	(±.007)	(±.023)	(±.015)	(±.080)	(±.014)	(±.095)
MAY 24	0.918	1.816	0.963	1.323	1.037	1.576	0.918	1.763
	(±.029)	(±.033)	(±.015)	(±.014)	(±.015)	(±.026)	(±.014)	(±.038)

Table 4. Means and standard errors of chlorophyll a (Chl-a) and phaeopigment (Phaeo) concentrations (μg/l) from sampled dates for treatments and control (Summer Experiment #2, July 19 - August 17, 1991). Each value represents an average of three replicates. Treatments: F+A= salmon effluent and 18 g/l of <u>Palmaria mollis</u>; F-A= salmon effluent alone; R+A= ambient seawater and 18 g/l of <u>P. mollis</u>; R-A= ambient seawater alone (control).

SAMPLING DATE	F+A		F-A		R+A		R-A	
	Chl-a	Phaeo	Chl-a	Phaeo	Chl-a	Phaeo	Chl-a	Phaeo
JULY 6	2.575	6.566	2.558	5.216	2.491	7.130	2.322	7.613
	(±.000)	(±.083)	(±.016)	(±.311)	(±.067)	(±.183)	(±.050)	(±.096)
AUG 13	12.030	15.826	10.180	39.606	12.666	50.293	11.603	87.183
	(±.626)	(±.653)	(±.121)	(±1.566)	(±.626)	(±2.043)	(±.078)	(±1.885)
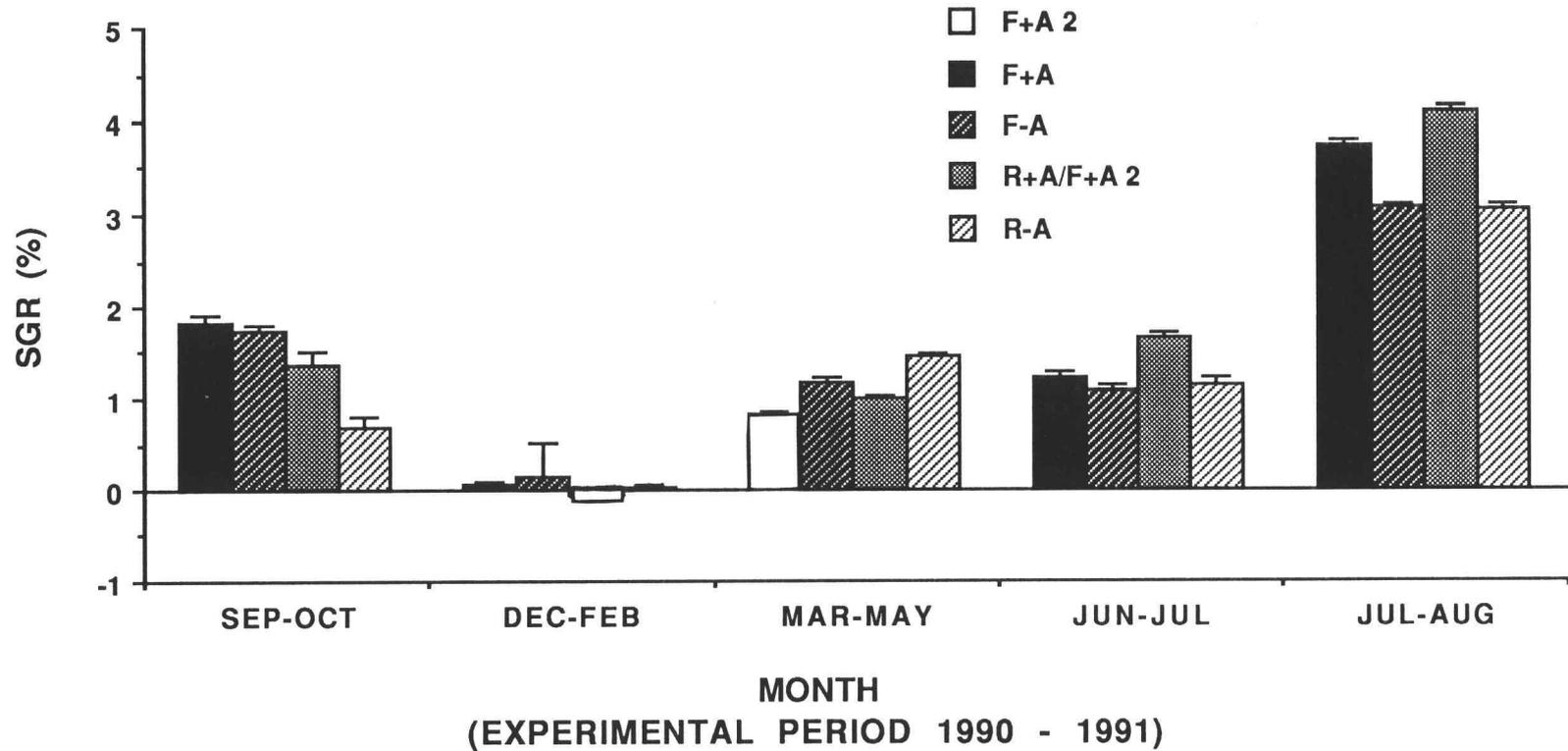
FIGURE 10.- Seasonal variation of mean specific growth rate (SGR) for juvenile Pacific oysters, experimental period: September 7, 1990 – August 17, 1991. Error bars represent the standard errors of the means. Legend: F+A= salmon effluent conditioned by <u>Palmaria</u> <u>mollis</u>; F-A= salmon effluent alone; R+A= ambient seawater conditioned by <u>P</u>. <u>mollis</u>, and R-A= ambient seawater alone (control). During Winter and Spring Experiments R+A was substituted by F+A 2= salmon effluent conditioned by twice the stocking density of alga that results in maximum algal yield for December and March, respectively, based on light availability, after Levin (1990).

the salmon effluent, while salmon effluent conditioned by the addition of macroalga significantly improved oyster and clam growth during summer (Fig. 11). Salmon effluent alone or salmon effluent conditioned by macroalga did not improve oyster growth in winter or spring. Furthermore, growth was significantly greater ($P<0.001$) in the control (R-A) than in any of the other treatments during spring, 1991.

Detailed information on growth for each experiment, will be discussed separately following the same seasonal sequence given in Materials and Methods.

Percentage mortality at each sampling period, per treatment, is presented in Table 5. Mortality was low throughout the whole experimental period. Highest mortality was 5% in the Winter Experiment and during Summer Experiment #1 for oysters in the R-A treatment. Lowest mortality (0%) occurred during Summer Experiment #2 for oysters in the F+A and F-A treatments and for clams in F-A and R+A treatments during the same season. Overall highest mortalities (regardless of the treatment) occurred in winter. Overall lowest mortalities occurred in fall and during Summer Experiment #2.
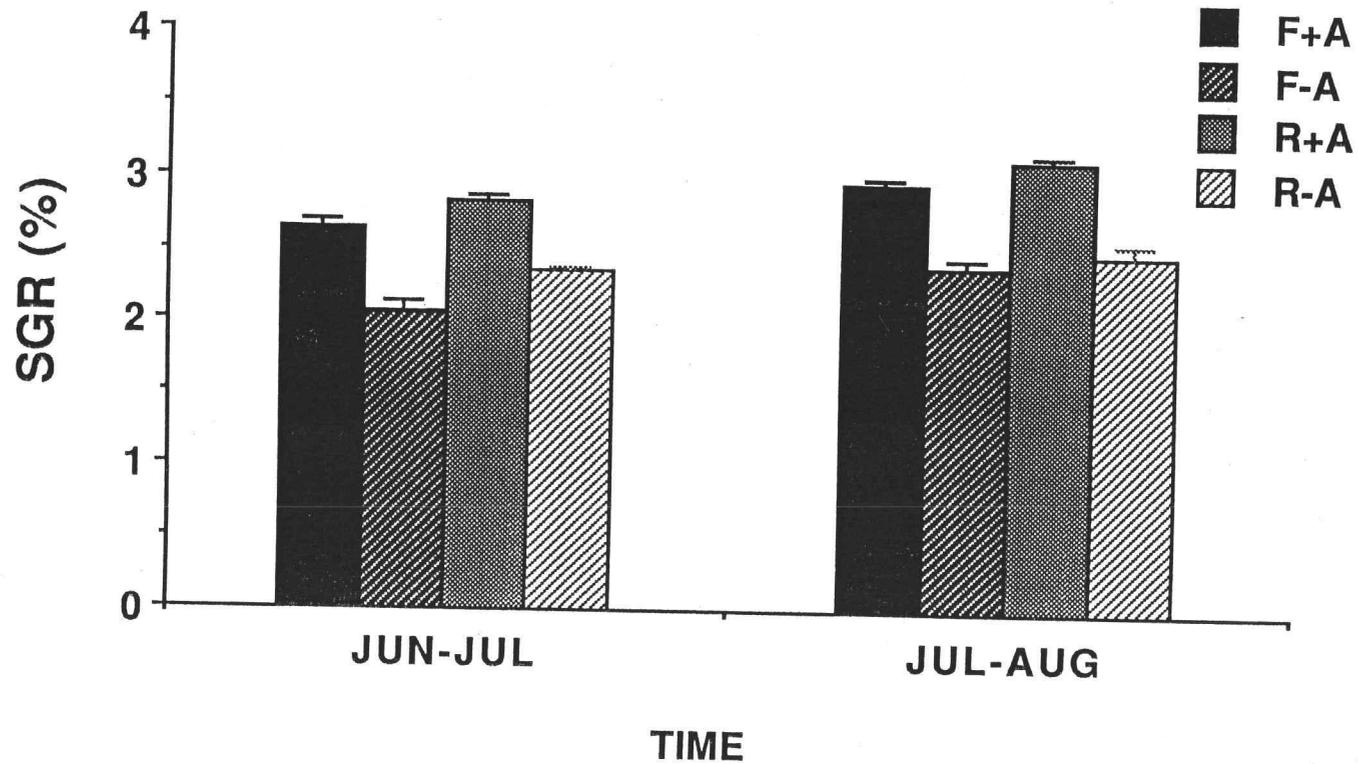
FIGURE 11.- Mean total specific growth rates (SGR) and standard errors of means for juvenile Manila clams (n=3) during Summer Experiment #1 (June 3 - July 4, 1991) and Summer Experiment #2 (July 19, - Aug 17, 1991). Legend: F+A= salmon effluent conditioned by 18 g/l _Palmaria mollis_; F-A= salmon effluent alone; R+A= ambient seawater conditioned by 18 g/l _P. mollis_, and R-A= ambient seawater alone.

Table 5. Mortality of bivlaves (%) per treatment at the end of each experimental period. Treatments for each season are stated in the text.

FALL EXPERIMENT

Treatment	Average Number Of Animals Per Replicate (n=5)	% Mortality
F+A	127	1.6
F-A	125	0.3
R+A	126	0.2
R-A	130	1.5

WINTER EXPERIMENT

Treatment	Average Number Of Oysters Per Replicate (n=8)	% Mortality
F+A2	137	3.0
F+A	157	3.0
F-A	153	3.0
R-A	134	5.0

SPRING EXPERIMENT

Treatment	Average Number Of Animals Per Replicate (n=6)	% Mortality
F+A2	51	0.7
F+A	52	1.2
F-A	52	2.0
R-A	51	1.6

Table 5.
(continued) Mortality of bivlaves (%) per treatment at the end of each experimental period.

SUMMER EXPERIMENT #1

	OYSTERS		CLAMS	
TREATMENT	Average Number Of Animals Per Replicate (n=3)	% Mortality	Average Number Of Animals Per Replicate (n=3)	% Mortality
F+A	85	3.5	326	0.8
F-A	82	3.7	316	1.3
R+A	80	1.7	329	1.2
R-A	84	5.2	325	1.0

SUMMER EXPERIMENT #2

	OYSTERS		CLAMS	
TREATMENT	Average Number Of Animals Per Replicate (n=3)	% Mortality	Average Number Of Animals Per Replicate (n=3)	% Mortality
F+A	66	0.0	51	2.0
F-A	66	0.0	47	0.0
R+A	72	1.0	49	0.0
R-A	56	0.6	47	1.4

Fall Experiment

Mean individual live weight of oysters cultured in F+A and F-A treatments was twice as high as that of the control (Fig. 12). Growth rates of oysters in the F+A and F-A treatments were almost 3 times higher than those in the R-A treatment (Fig. 13). Growth rates in R+A treatment were twice as high as those in the R-A treatment; mean live weights of oysters in the latter treatment was 30% higher than mean live weights of oysters in the control. A two way ANOVA revealed a highly significant positive effect of the salmon effluent on mean SGR (P=0.007) and mean live weights (P=0.001) of oysters. There was also a significant positive effect of $\underline{P}$. $\underline{mollis}$ on mean live weights (P=0.03) and SGR (P=0.004) of bivalves. There was no significant interaction between factors. There was a highly significant positive effect of the salmon effluent (P<0.001) on organic weights of oysters at the end of this experiment. Mean organic weights of oysters raised in the salmon effluent treatments (F+A and F-A) were twice as high as those in the control and 30% higher than organic weights in the R+A treatment (Fig. 14). No significant effect of the alga was found on organic weights of oysters. Average water temperature during September was 16°C and steadily decreased to 13°C during the last week of October, when the Fall Experiment ended.
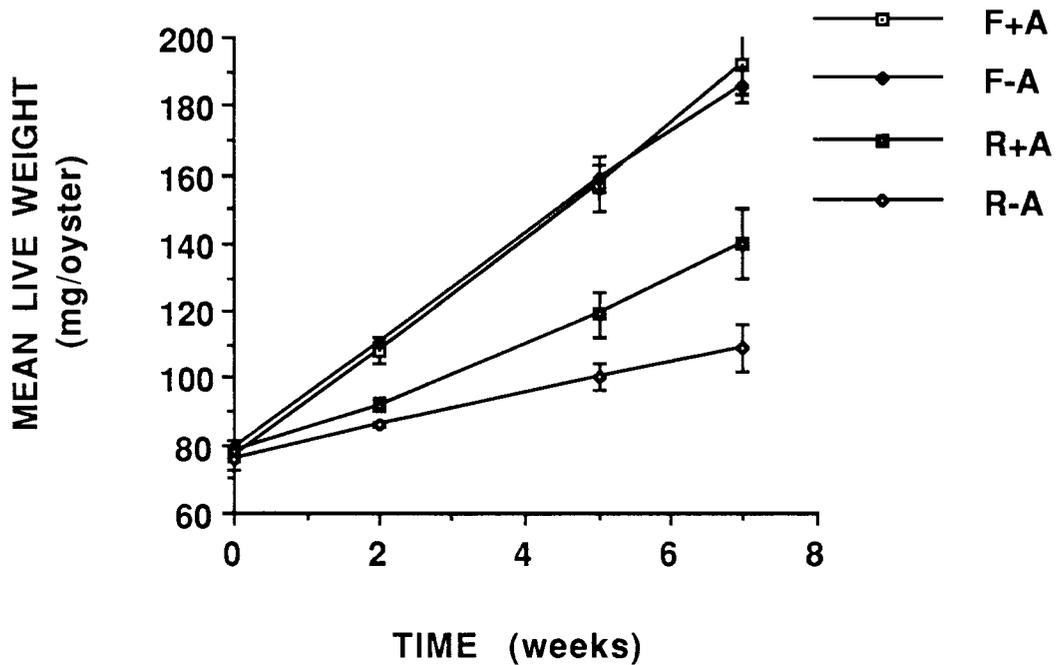
FIGURE 12.- Mean individual live weight (mg/oyster) of juvenile Pacific oysters, Fall Experiment (Sep 7 -Oct 31, 1990). Error bars represent standard errors of the means (n=5). Legend: F+A= salmon effluent conditioned by 10.4 g/l *Palmaria* *mollis*; F-A= salmon effluent alone; R+A= ambient seawater conditioned by 10.4 g/l of P. *mollis*, and R-A= ambient seawater alone (control).
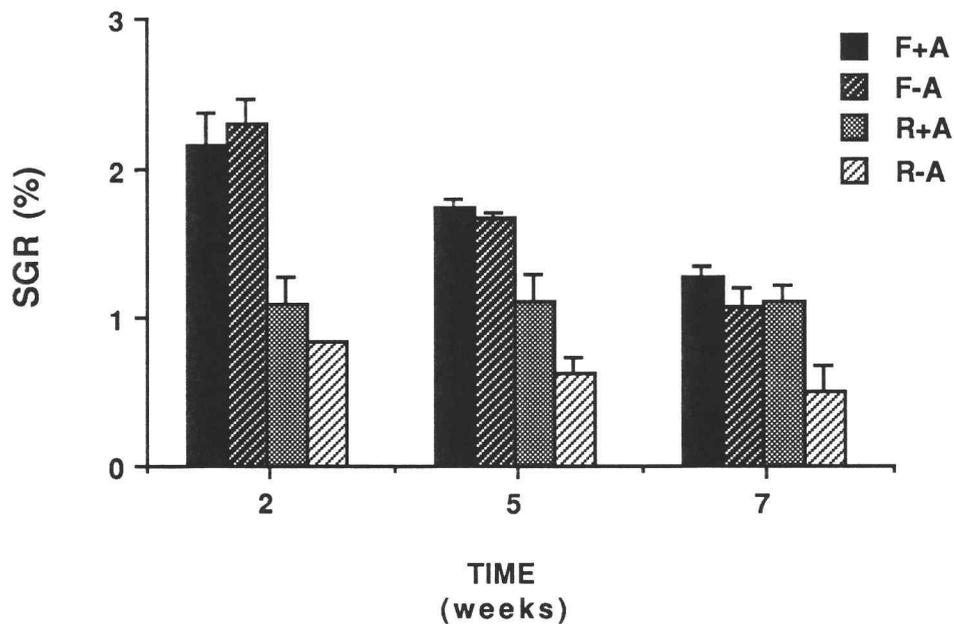
FIGURE 13.- Mean specific growth rate (SGR) of juvenile Pacific oysters, Fall Experiment (Sep 7 - Oct 31, 1990). Error bars represent standard errors of the means (n=5). Legend: F+A= salmon effluent conditioned by 10.4 g/l _Palmaria_ _mollis_; F-A= salmon effluent alone; R+A= ambient seawater conditioned by 10.4 g/l _P_. _mollis_; R-A= ambient seawater alone (control).
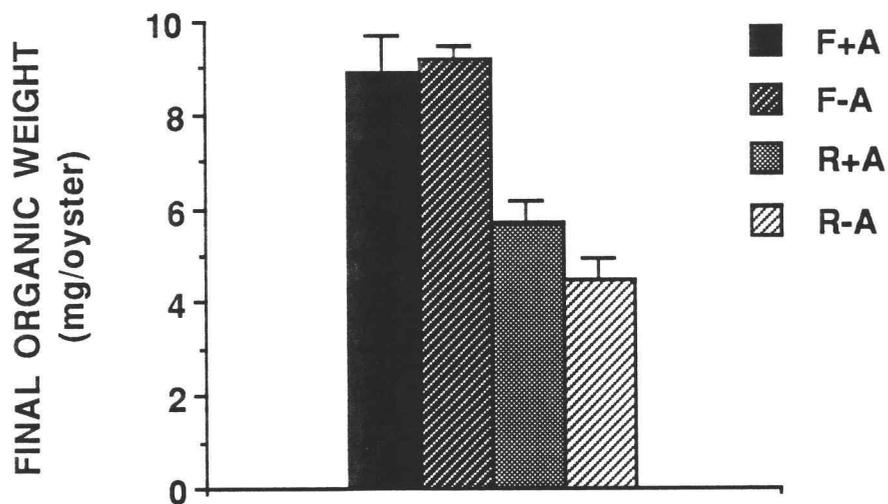
FIGURE 14.- Final mean organic weights and standard errors of the means (n=5) for juvenile Pacific oysters at the end of the Fall Experiment (Sep 7 - Oct 31, 1990). Legend: F+A = salmon effluent conditioned by 10.4 g/l <u>Palmaria</u> <u>mollis</u>; F-A= salmon effluent alone; R+A= ambient seawater conditioned by 10.4 g/l <u>P</u>. <u>mollis</u>; R-A= ambient seawater alone (control).

Winter Experiment

No statistically significant differences were found in growth of oysters from various treatments during this experiment (Fig. 15). Growth rates showed great variability throughout the experiment; poor initial physiological condition of $\underline{P}$. $\underline{mollis}$ stocked in the experimental tanks and extremely low water temperatures were probably responsible for this variation. Due to the variability introduced by these factors, growth could not be statistically analyzed by standard parametric procedures used in other experiments. SGR's were the lowest recorded for any experiment performed, ranging from SGR = - 0.14 in F+A2 treatment (the lowest) during the first weighing, to SGR = 0.28 in F-A treatment (the greatest) during the last measurement (Fig. 16). Even though no particular trends could be assessed from the results of this experiment, growth rates of the oysters in the control and in F+A treatment seemed to steadily decrease with time; F+A2 and F-A showed an inconsistent pattern. F+A2 provided negative growth rates during the first and second weighing and seemed to recover during the last measurement. F-A treatment always showed higher growth rates than those of the control, especially at the end of the experiment. Non-parametric analyses showed that SGR's of oysters in the F-A treatment were significantly higher (P=0.001) than SGR's of oysters grown
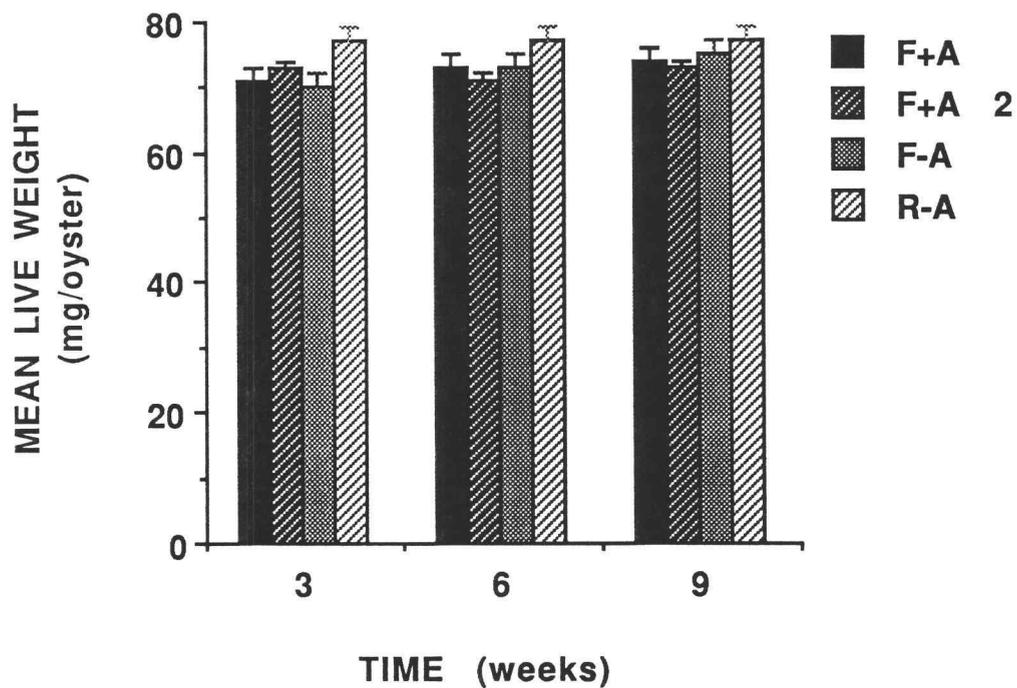
FIGURE 15.- Mean total live weights and standard errors of the means (n=8) for juvenile Pacific oysters, Winter Experiment (Dec 7, 1990 - Feb 15, 1991). Legend: F+A= salmon effluent conditioned by 7 g/l <u>Palmaria</u> <u>mollis</u>; F+A2 = salmon effluent conditioned by 12 g/l <u>P</u>. <u>mollis</u>; F-A= salmon effluent alone; R-A= ambient seawater alone (control).
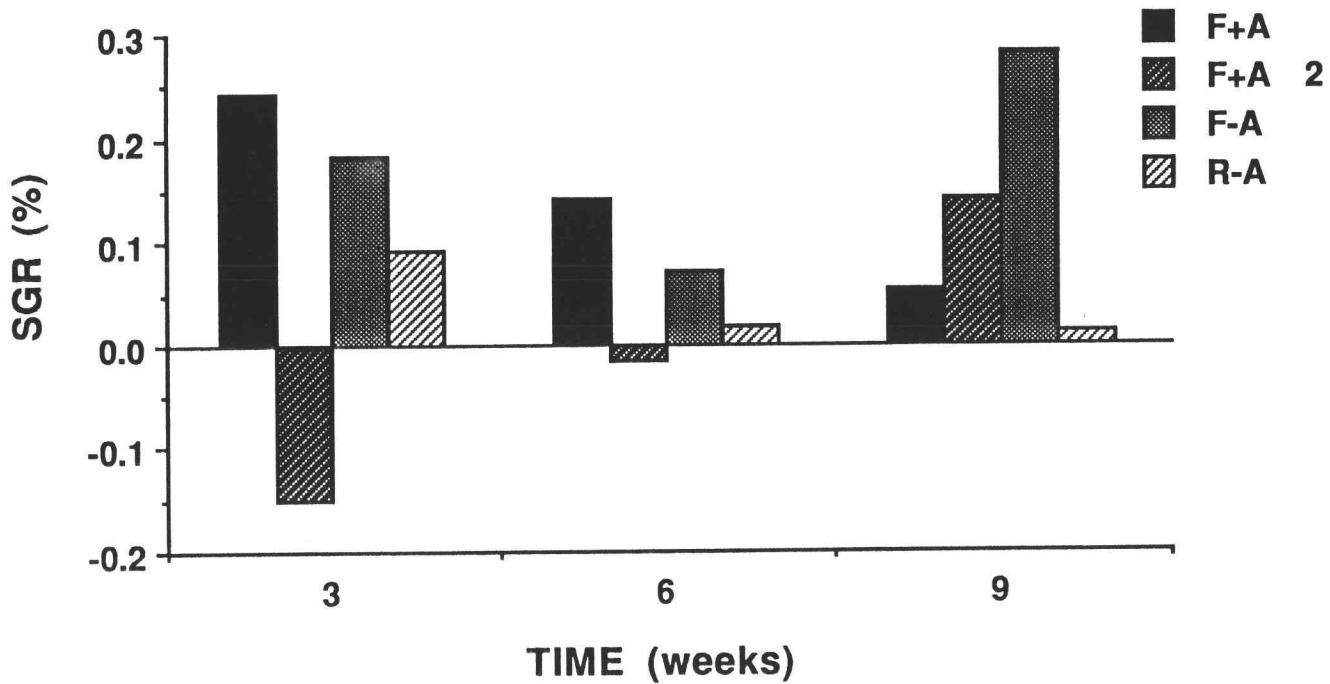
FIGURE 16.- Mean specific growth rate (SGR) and standard errors of the means (n=8) for juvenile Pacific oysters during Winter Experiment (Dec 7, 1990 – Feb 15, 1991). Legend: F+A= Salmon effluent conditioned by 7 g/l <u>Palmaria mollis</u>; F+A 2= salmon effluent conditioned by 12 g/l <u>P</u>. <u>mollis</u>; F-A= salmon effluent alone; R-A= ambient seawater alone (control).

in the control at the end of the experiment. No data on organic weights for the oysters were available for this experiment. Water temperatures were lowest during this season, ranging from 7°C to 12°C.

Spring Experiment

Mean live weights and SGR's of oysters were significantly higher (P<0.001) in the control (R-A) than in any of the other treatments for this experiment (Fig. 17 and 18). No significant differences were found in mean individual live weights of oysters among the treatments with alga (F+A and R+A). The F-A treatment provided significantly higher growth rates than F+A and R+A treatments (P<0.001). Organic weights of the oysters were significantly lower (about 50% less) in F+A and R+A treatments (P<0.001) than those in the F-A or R-A treatments. The lowest means for both mean live weight and SGR were those of oysters in the F+A2 treatment. No significant difference was found in the organic weights of oysters in the F-A treatment when compared to those in the R-A treatment (Fig. 19).

SGR's varied greatly throughout the experimental period. Highest SGR's were recorded during the second and fourth measurements (after the fourth and eight weeks of experimentation).
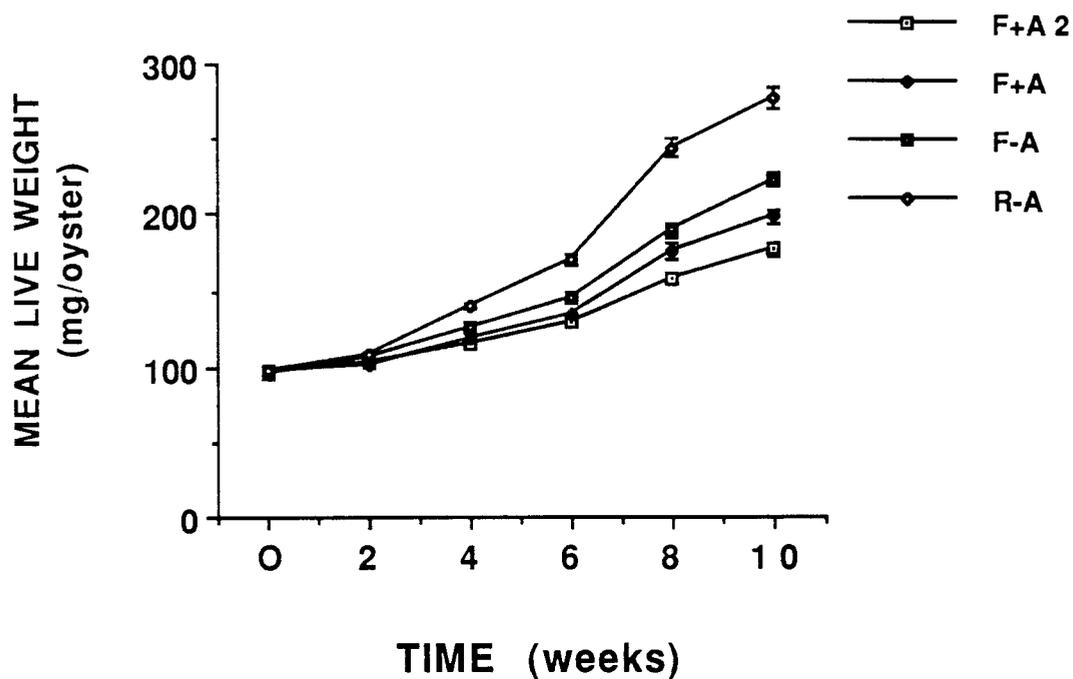
FIGURE 17.- Mean individual live weight (mg/oyster) and standard errors of the means (n=6) for juvenile Pacific oysters, Spring Experiment (March 7 - May 24, 1991). Legend: F+A2= salmon effluent conditioned by 40 g/l Palmaria mollis; F+A= a mixture of salmon effluent conditioned by 40 g/l P. mollis and ambient seawater (50%-50%); F-A= salmon effluent alone and R-A= ambient seawater alone (control).
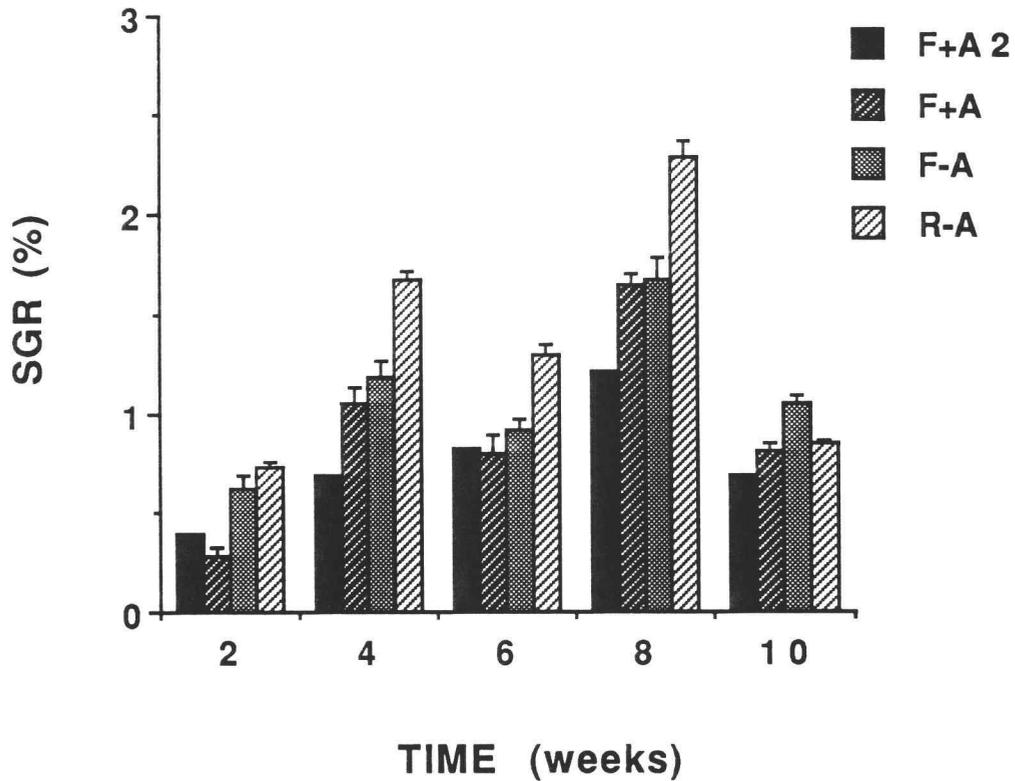
FIGURE 18.- Mean specific growth rate (SGR) and standard errors of the means for juvenile Pacific oysters (n=6), Spring Experiment (March 7 - May 24, 1991). Legend: F+A2= salmon effluent conditioned by 40 g/l <u>Palmaria</u> <u>mollis</u>; F+A = a mixture of salmon effluent conditioned by 40 g/l <u>P</u>. <u>mollis</u> and ambient seawater (50%-50%); F-A= salmon effluent alone, and R-A= ambient seawater alone (control).
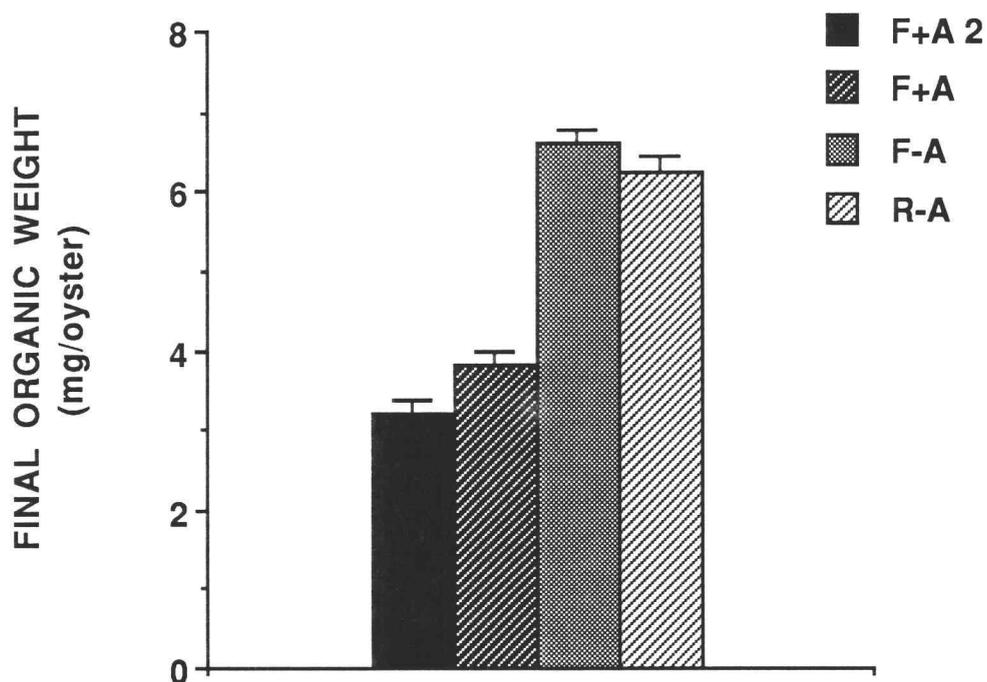
FIGURE 19.- Final mean organic weights and standard errors of the means (n=6) for juvenile Pacific oysters at the end of Spring Experiment (March 7 - May 24, 1991). Legend: F+A2= salmon effluent conditioned by 40 g/l <u>Palmaria mollis</u>; F+A = a mixture of salmon effluent conditioned by 40 g/l <u>P. mollis</u> and ambient seawater (50%-50%); F-A= salmon effluent alone, and R-A= ambient seawater alone (control).

After winter, the coldest temperatures were those recorded in spring ranging from a minimum of 10°C to a maximum of 12.5°C from March through May.

Summer Experiment #1

Results on mean individual live weights, SGR's and organic weights of oysters and clams for this experiment are presented in figures 20-22.

There was an 80% reduction in the flow rates of F-A treatment during most of the duration of this experiment, due to blockage of the drains of the experimental units containing animals. The cause of the blockage was an excess of organic matter generated in the salmon tank that passed to the water reservoirs and then to the experimental units. Even though these were meticulously cleaned and flushed every day, as the days became longer and sunnier, the problem worsened. In the mornings, after the material had accumulated overnight, water exchange rates in the F-A treatment were significantly lower than those in other treatments. It was observed that F+A treatment experienced almost no reduction in flow rate because the alga caused settlement of the clumps of suspended material from the fish tank. As a result, mean live weights (P=0.002), SGR (P=0.0003) and organic weights (P=0.0088) of oysters in the F-A treatment were significantly lower than in any of the other treatments.
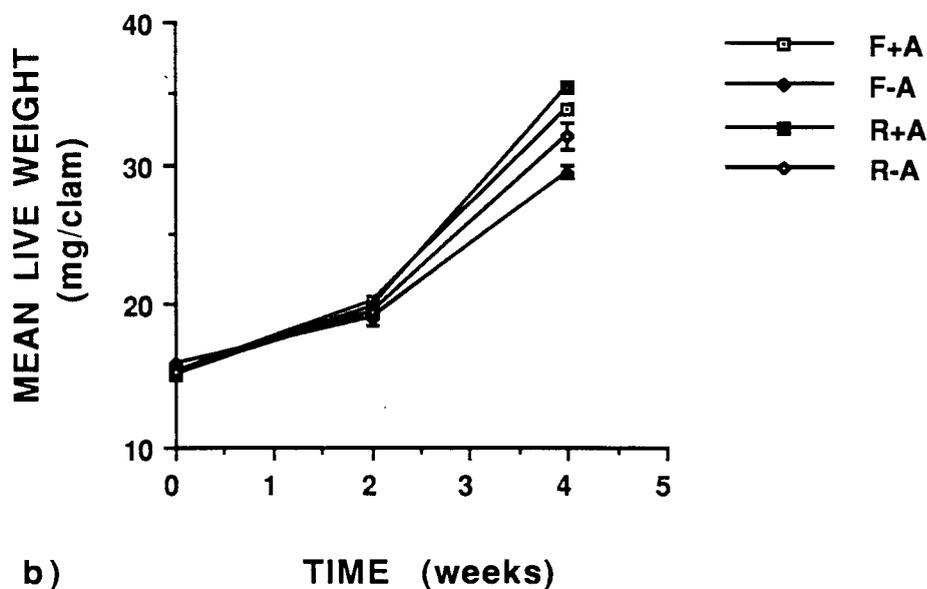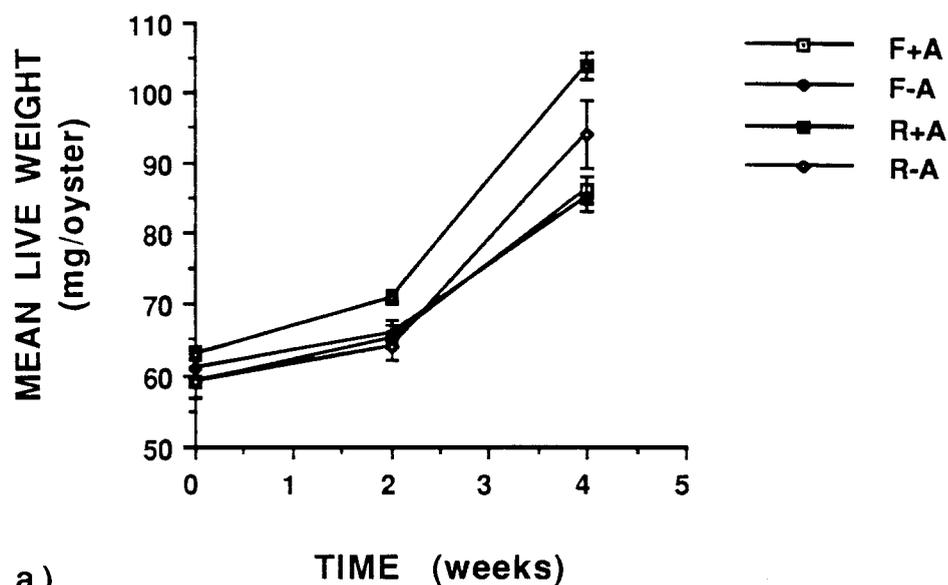
FIGURE 20.- Mean individual live weight and standard errors of the means (N=3) for: a) juvenile Pacific oysters, and b) Manila clams, Summer Experiment #1 (June 3 - July 4, 1991). Legend: F+A= salmon effluent conditioned by 18 g/l Palmaria mollis; F-A= salmon effluent alone; R+A= ambient seawater conditioned by 18 g/l P. mollis, and R-A= ambient seawater alone (control).
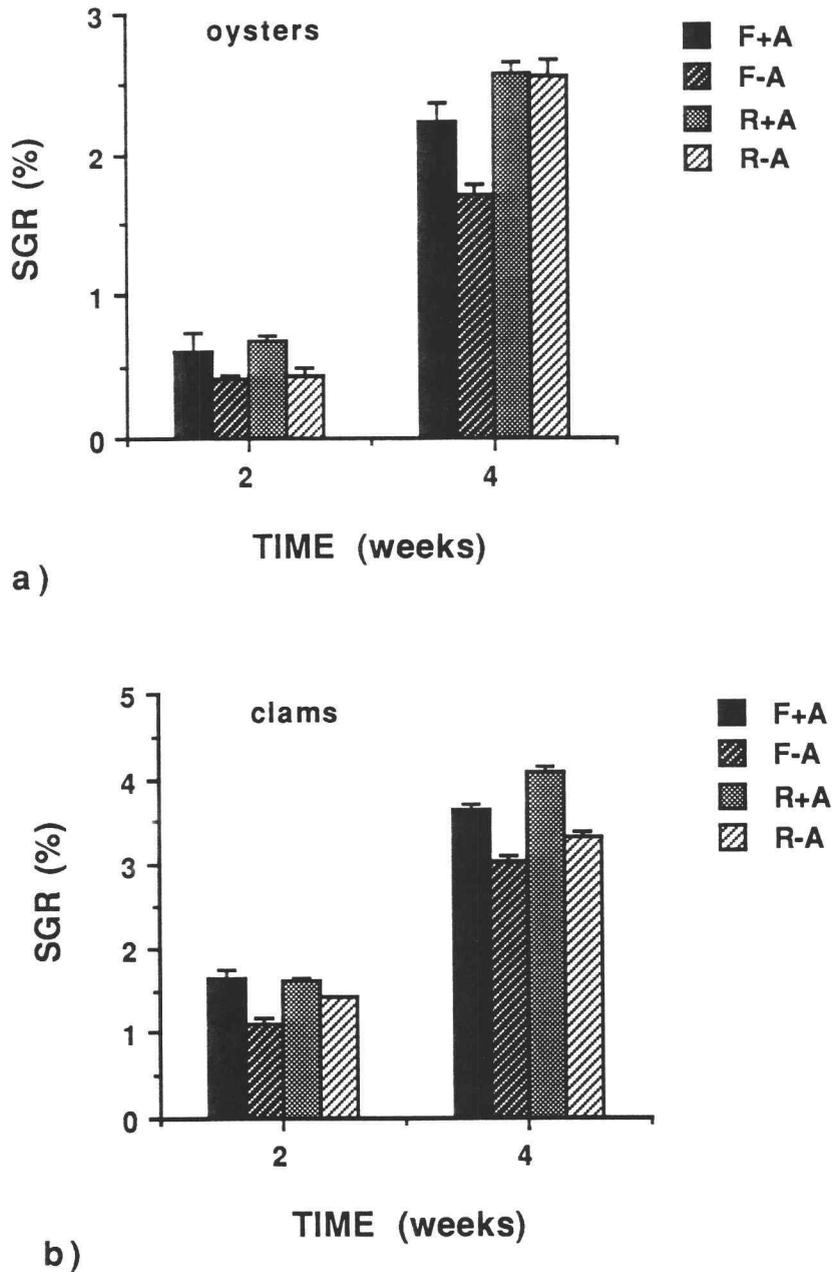
FIGURE 21.- Mean specific growth rate (SGR) and standard errors of the means (N=3) for: a) juvenile Pacific oysters, and b) Manila clams, Summer Experiment #1 (June 3 - July 4, 1991). Legend: F+A= salmon effluent conditioned by 18 g/l Palmaria mollis; F-A= salmon effluent alone; R+A= ambient seawater conditioned by 18 g/l P. mollis, and R-A= ambient seawater alone (control).
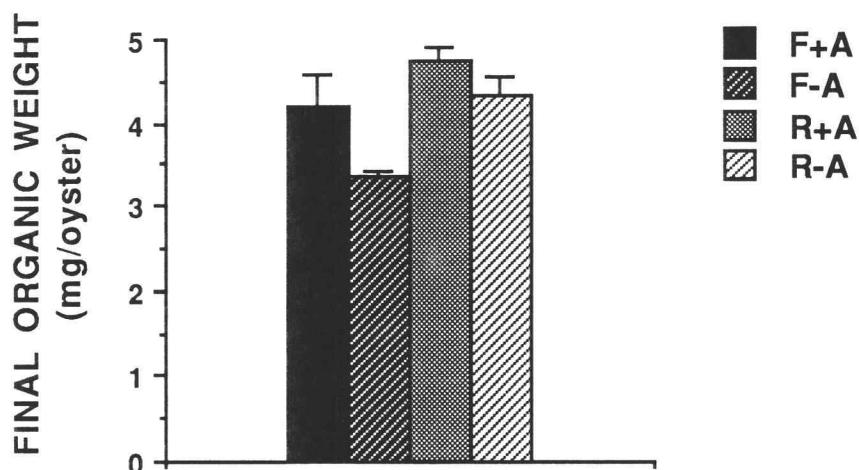
a)
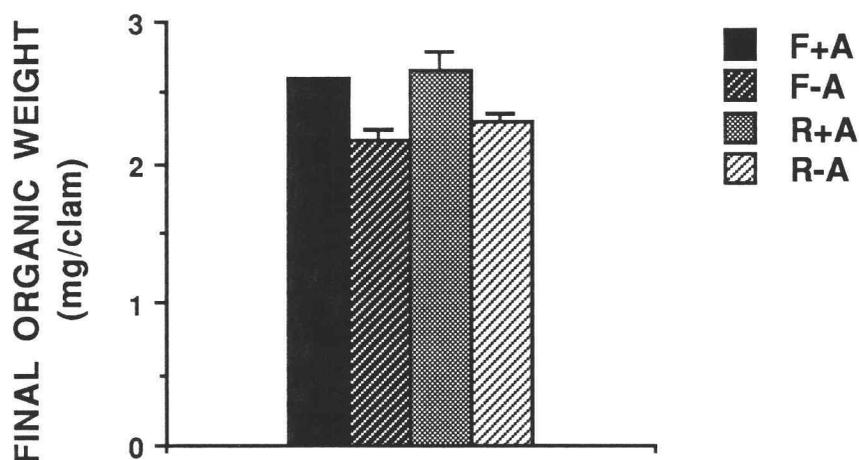
b)

FIGURE 22.- Final mean organic weight and standard errors of the means (N=3) for: a) juvenile Pacific oysters, and b) Manila clams, Summer Experiment #1 (June 3 - July 4, 1991). Legend: F+A= salmon effluent conditioned by 18 g/l of _Palmaria_ _mollis_; F-A= salmon effluent alone; R+A= ambient seawater conditioned by 18 g/l _P_. _mollis_, and R-A= ambient seawater alone (control).

The salmon effluent also had a significant negative effect on mean weights (P=0.0124) and SGR (P=0.0008) of clams. There was a significant positive effect of the alga on growth rates (P=0.0125) and organic weights (P=0.0252) of oysters at the end of the experiment. There was also a highly significant positive effect of the alga on mean live weights (P=0.001), SGR (P<0.001) and organic weights (P=0.0005) of clams. Problems caused by unequal water flows described above were solved by modifying the system. The experiment was repeated in July (this was Summer Experiment #2).

Summer Experiment #2

The same apparatus utilized in Summer Experiment #1 was used for this experiment. To solve the problem of blockage, a sieve was installed in each one of the water reservoirs to prevent organic material from blocking the experimental units. Also, the screens that had been placed at the drain of each experimental unit, to keep the animals from being swept out of the outflow, were removed and larger animals were used.

The analyses of variance performed at the beginning of this experiment showed statistically significant differences in the initial weights of the oysters among the different treatments (P=0.0245). Due to those differences, organic and mean live weights of oysters were

not statistically analyzed (since there were only 3 replicates per treatment, a non-parametric approach was not useful either). SGR's were statistically analyzed, since this parameter is independent of initial differences in weights.

The results of this experiment greatly resemble those of Summer Experiment #1, except that the trends are more accentuated (Fig. 23-25). There was a highly significant positive effect of P. mollis on SGR (P=0.0001) of oysters, and in mean live weights (P=0.003), SGR (P=0.0056) and organic weights (P=0.0252) of clams. There was no significant effect of the salmon effluent on SGR of either oysters or clams. Mean live weights of oysters were highest in the treatments with alga than in any of the other treatments.

Highest mean SGR's for both oysters and clams were recorded in the R+A treatment.
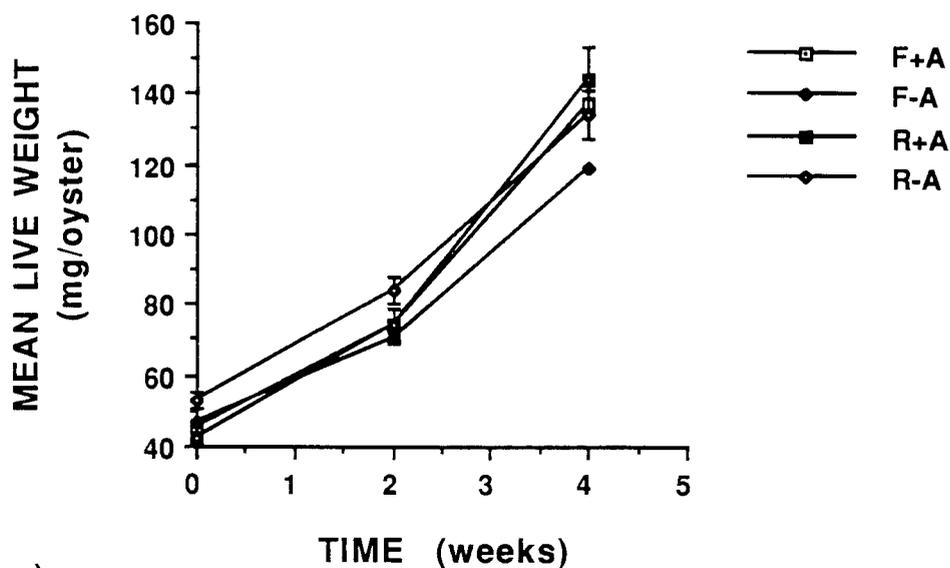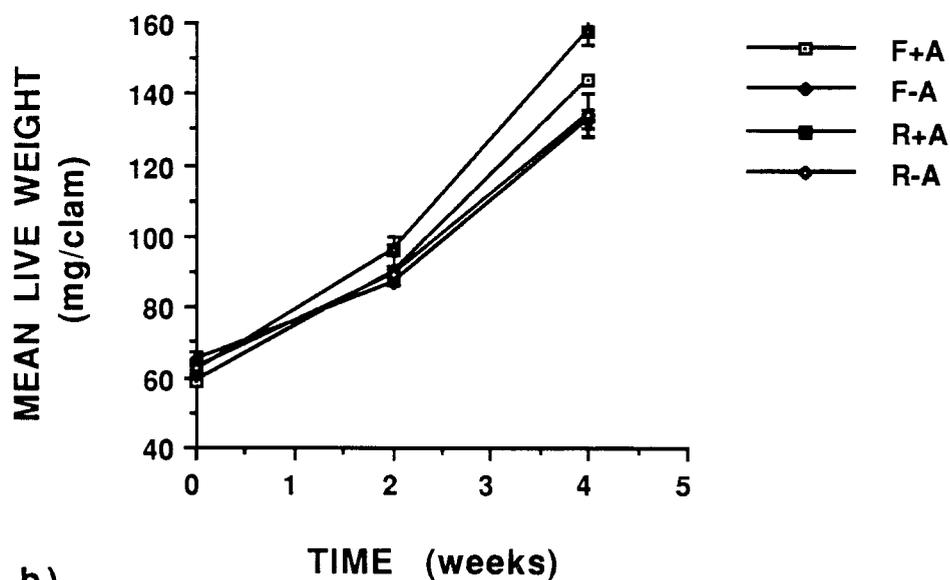
FIGURE 23.- Mean individual live weight and standard errors of the means (n=3) for: a) juvenile Pacific oysters, and b) Manila clams, Summer Experiment #2 (July 19 - Aug 17, 1991). Legend: F+A= salmon effluent conditioned by 18 g/l _Palmaria mollis_; F-A= salmon effluent alone; R+A= ambient seawater conditioned by 18 g/l P. _mollis_, and R-A= ambient seawater alone (control).
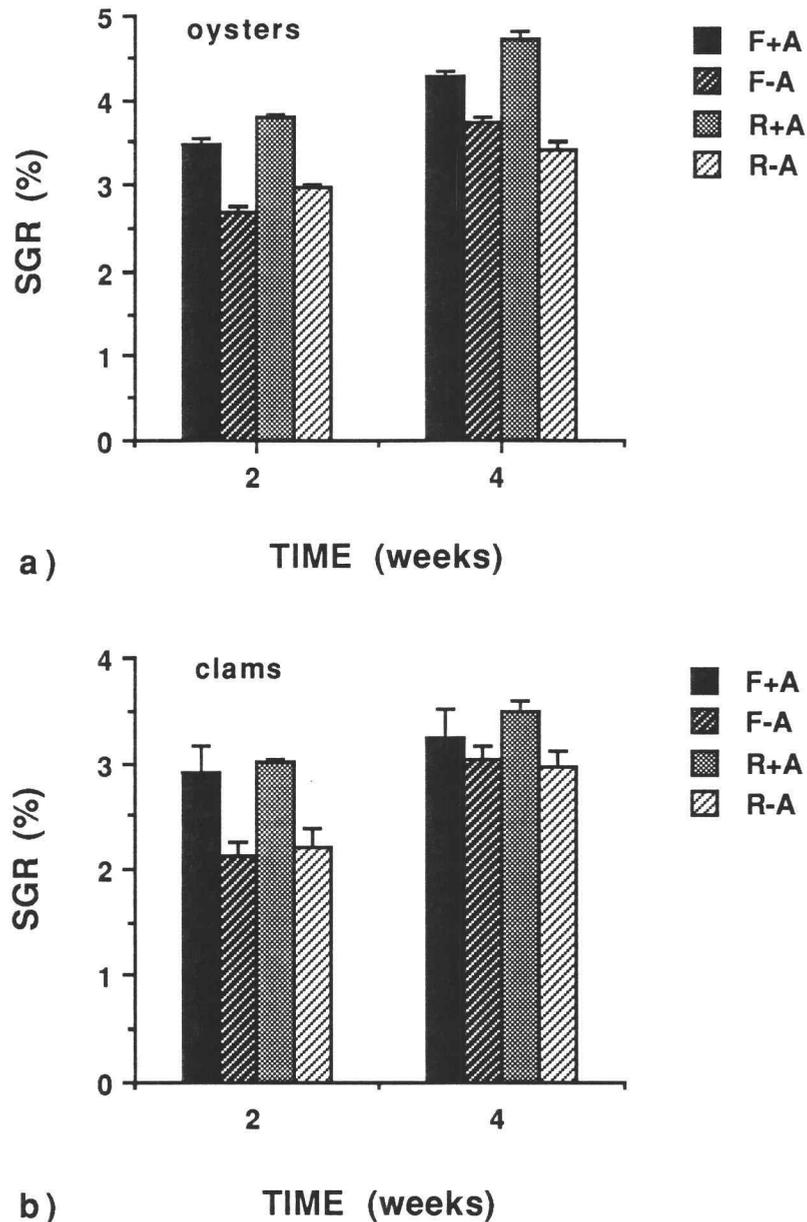
FIGURE 24.- Mean specific growth rate (SGR) and standard errors of the means (n=3) for: a) juvenile Pacific oysters, and b) Manila clams, Summer Experiment #2 (July 19 - Aug 17, 1991). Legend: F+A= salmon effluent conditioned by 18 g/l Palmaria mollis; F-A= salmon effluent alone; R+A= ambient seawater conditioned by 18 g/l P. mollis, and R-A= ambient seawater alone (control).
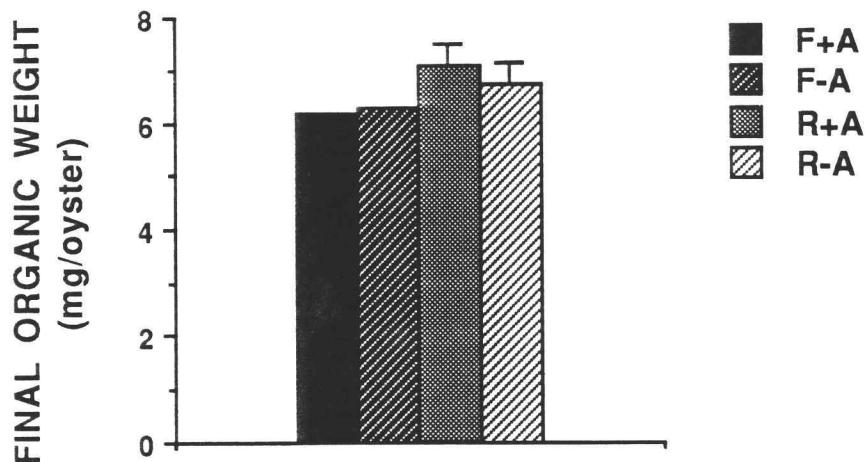
a)
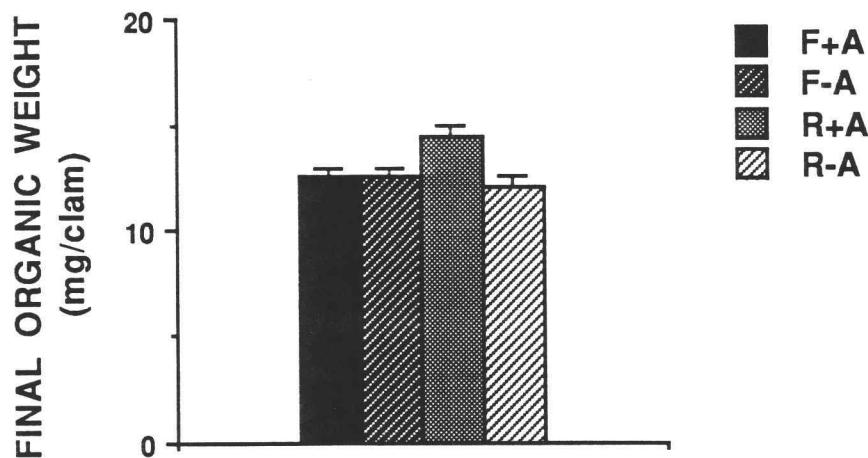
b)

FIGURE 25.- Final mean organic weight and standard errors of the means (n=3) for: a) juvenile Pacific oysters, and b) Manila clams, Summer Experiment #2 (July 19 - Aug 17, 1991). Legend: F+A= salmon effluent conditioned by 18 g/l <u>Palmaria</u> <u>mollis</u>; F-A= salmon effluent alone; R+A= ambient seawater conditioned by 18 g/l <u>P</u>. <u>mollis</u>, and R-A= ambient seawater alone (control).

DISCUSSION

Growth of juvenile Pacific oysters and Manila clams during the present study seemed to respond to the combined effects of the treatments tested and those of seasonal variables.

Among the many factors that affect bivalve growth, water temperature and food availability are probably the most important (Bayne and Newell, 1983; Breese and Malouf, 1977; Walne, 1972). It is likely that bivalve growth rates reported in this study were the result of the combined effects of differences in the food contained in tested treatments and temperature.

During this study, seasonal changes of environmental variables seemed to have a stronger impact on bivalve growth than did the tested treatments. Because changes in environmental variables affect both bivalve growth and primary productivity (Bayne et al., 1976, Ryther and Yentsch, 1957), the results obtained here will be discussed both in terms of the treatments and in terms of seasonal changes of the environmental variables, especially temperature.

Our results on growth of Pacific oysters during this study, agree with those of Malouf and Breese (1977) in that minimal growth was achieved from November through March and in that growth rates were highest in July and

August, regardless of the treatment. Malouf and Breese (1977) reported profound seasonal changes in growth rates of immature Pacific oysters, in water pumped from Yaquina Bay, that were also evident in the present study.

During fall, natural productivity in ambient seawater tends to decrease as a result of diminished daylight and upwelling that characterizes coastal waters of the Pacific Northwest (Cushing, 1956; NOAA, NMSF 1990, 1991). It seems reasonable to suggest that during this time, the oysters would depend to a greater extent on nutrients provided in the salmon and macroalga effluents as a source of food than on natural phytoplankton. The salmon effluent is rich in particulate organic carbon and other nutrients that could have been utilized directly by both macroalga and bivalves for growth (Foy and Rossel, 1991). It still remains to be determined, though, whether the benefits of the salmon effluent and P. mollis on growth of the Pacific oyster were due to direct utilization of particulate organic matter (POM), dissolved organic matter, (DOM) or to an increased primary productivity resulting from the eutrophication of the water.

Phytoplankton is undoubtly the primary source of organic carbon in much of the world oceans (Duggins, 1989). The point of view that phytoplankton provides the main source of food for bivalve mollusks has gained wide acceptance (Hartman et al., 1973). Malouf and Breese

(1978) demonstrated that seasonality of growth in oysters is greatly related to changes in available food. Oyster and clam growth, in particular, has often been correlated with high primary productivity (Jones and Iwama, 1991; Brown and Hartwick, 1988; Utting, 1988). Even though no chlorophyll data are available for Fall Experiment, it is likely that the oysters may have derived part of their diets from that source. Brown diatoms were seen to develop in the polyethylene cells that delivered water to each upweller, possibly due to a combination of high water temperatures and nutrient availability from the waste products contained in the salmon and macroalga effluents. Waste products generated from salmon farming have been proven to promote higher growth rates in oysters, by direct utilization of POM and by increased phytoplankton concentrations due to higher nutrient availability in the environment (Ryther et al., 1974; Jones and Iwama, 1991).

Results from the Winter Experiment during the present study agree with those of Malouf and Breese (1977) who reported little or no growth of juvenile Pacific oysters in Yaquina Bay water during this season. In our study, the high variability in growth was due to extremely bad weather conditions (very low water temperatures) and to poor quality of P. mollis initially stocked in the experimental tanks. However, it was clear that no great significant improvement in the growth of oysters was

achieved in any of the treatments during this season. Growth rates of oysters in the F-A treatment were significantly higher than those of the control, suggesting that there might be a positive effect of the salmon effluent on growth of Pacific oyster during this time of the year, when primary productivity in the ambient seawater is normally low. During winter, however, the oysters could not take advantage of this "extra food" provided by the salmon and macroalga effluents because low temperatures were severely limiting growth.

In spring, warmer water temperatures combined with abundant suspended detritus in the seawater, and higher primary productivity values than in winter may have contributed to the oyster's higher growth rates. Upwelling areas, like the Pacific Northwest, are usually associated with high primary productivity (Broecker and Peng, 1982), so it's not unlikely that phytoplankton blooms during spring supported the higher growth rates of oysters in the spring experiment. High concentrations of phaeopigments, indicating the presence of detritus and/or dead organic matter, were found to be more abundant in ambient seawater than in any other treatment in Spring and Summer Experiments. It seems, from the particulate organic sample analyses carried out in summer, that the alga removed some POM from the incoming effluent in treatments with $\underline{P}$. $\underline{mollis}$ (F+A and R+A treatments had

lower POM concentrations than F-A or R-A). This might explain why bivalve growth rates were significantly less in those treatments: because chlorophyll values were fairly low in spring, POM was likely to be used as a complementary source of food by oysters. Since some POM from the salmon effluent was removed by the macroalgae, less food was available for the bivalves in F+A and R+A treatments .

Salmon effluent stocked with high densities of $\underline{P}$. $\underline{mollis}$ has been demonstrated to result in low growth rates of Manila clams, (Levin, pers. comm.). Our results agree with these findings, since growth rates of oysters during the Spring Experiment were significantly lower in the treatment containing twice the recommended stocking density of $\underline{P}$. $\underline{mollis}$ for that time of the year. A similar trend was observed in the Winter Experiment, when negative growth was recorded for oysters in the F+A2 treatment.

Higher growth rates of bivalves in the two Summer Experiments are probably the result of a combination of factors. There were higher concentrations of phytoplankton present in the water compared to concentrations recorded earlier in spring. In addition, water temperatures were also warmer in summer than those in winter and spring.

It has been demonstrated that low concentrations of silt or inorganic particulate materials can increase

feeding rates of lamellibranchs by mechanically stimulating the gills (Winter, 1977, Loosanoff, 1962; Urban and Langdon, 1984). Concentrations of inorganic suspended matter were higher in ambient seawater than in any of the other treatments during summer, and this may have also contributed to the higher growth rates of the bivalves, compared to growth during other seasons.

It is noteworthy that the R+A treatment and not the F+A treatment, as one would logically expect from the results in Fall Experiment, yielded the highest bivalve growth rates in summer. This suggests that the ambient seawater was richer in food in summer than in Fall or Winter Experiments. The particulate suspended matter analyzed from the water samples in Summer Experiment #1 contained organic material that the oysters could have utilized as food. The fraction of particulate organic matter in the ambient seawater during this experiment was just as high as that in the F+A treatment, adding evidence that during this time of the year the ambient seawater was as rich in food as the salmon effluent. Then, during spring and summer, when primary productivity normally increases, bivalve growth may not be improved by the utilization of salmon effluent, since the ambient seawater is rich enough in nutrients and phytoplankton to produce high growth rates.

The salmon effluent did not improve bivalve growth in

summer. This may be due in part to a low density of salmon that provided the salmon effluent for Summer Experiments #1 and #2. The stocking density of salmon was half that used in fall of 1990, because of fish mortalities that were not replaced. The reduced fish biomass may have resulted in nutrient levels well below optimum concentrations for optimum bivalve growth. An alternative explanation is that since food was abundant in ambient seawater in summer there was plenty of food in all water treatments, but because temperature was significantly higher in treatments with alga, bivalves in treatments containing P. mollis had significantly greater growth rates than those cultured in salmon effluent or ambient seawater alone.

The effect of the temperature on growth rates of Pacific oysters seems to be evident as growth rates decreased steadily when winter approached and temperature decreased from an average of 16°C in September to 13°C at the end of October. It is noteworthy that growth rates of oysters were more consistent throughout the experiment in treatments with alga than in treatments without it, which is further evidence of the "warming" effect of the alga (growth rates in treatments with alga did not decrease as rapidly as growth rates in the other treatments because temperatures were warmer and helped to generate more stable growth).

Further evidence of the effect of temperature and higher phytoplankton levels on the growth of bivalves is furnished by close examination growth during the different seasons. In fall, growth (increase in live weight) was almost linear, and even became slightly asymptotic at the end of the experiment. During the first weeks of the Spring Experiment, it started to show the exponential form that characterizes oyster growth at this early stage. Growth became clearly exponential in summer in all treatments, when food was abundant and temperatures were closest to the optimum for bivalve growth. This increased growth became even more pronounced during Summer Experiment #2, when solar radiation, primary productivity and temperature were highest.

During summer, growth rates of bivalves in ambient seawater were higher than in any other season. This suggests that natural food in ambient seawater was adequate in summer and deficient during the other seasons. Therefore, the observed differences in bivalve growth between treatments with and without alga in summer could be most likely due to a temperature differential and not to differences in the amount of food available. (Note that, for Summer Experiment #2, when greatest growth rates were recorded, water flow rates were highest and bivalve biomass smallest than in any other experiment, so food could not have limited growth then). In other words,

growth rates of bivalves in summer were high in all water treatments because food was abundant and temperature was closer to optimum for bivalve growth compared to that in winter or spring. Higher growth rates of oysters and clams in treatments with alga could well be due to warmer water temperatures in those treatments, since high growth is expected to result whenever a combination of high water temperatures and elevated levels of food occur (Bayne and Newell, 1983; Brown and Hartwick, 1988). Manzi et al. (1986), reported that greatest growth of juvenile clam, Mercenaria cultured in an upflow nursery system, occurred during fall, coinciding with highest chlorophyll concentrations (at optimum temperatures). Bourne (1982), found growth rates of Manila clam to be highly correlated with temperature changes. This effect of temperature on growth rates of Manila clams has been reported by other authors (Langton et al., 1977; Goulletquer et al., 1988).

Growth rates of Manila clams reported in this study in early summer agree with those found by Levin during a 5 week experiment made in June, 1991 (unpublished, pers. comm.). He used an upweller system to grow juvenile Manila clams in salmon effluent conditioned by a stocking density of P. mollis similar to that reported in this study. However, there is evidence that juvenile Manila clams can express highly variable growth rates when grown under different experimental conditions (Langton et al.,

1977; Bourne, 1982; Chew, 1989).

According to Levin (pers. comm.) salmon and macroalga effluents have reported to have a greater beneficial effect on Manila clam growth than those observed in this research. Besides differences in the culture apparatus and methodology used in my experiments and his, the disagreement between our results may be due to the fact that clams are more efficient in assimilating food than oysters are. Therefore, clams have a higher net production than oysters and others bivalves (Tenore et al., 1973).

CONCLUSIONS

Results of these experiments suggest that significant improvement in growth of juvenile C. gigas may be achieved when juveniles are grown in salmon effluent during fall, provided that water temperatures remain within a range close to the optimum for bivalve growth. Such temperatures were recorded in the present study during early fall and late summer. Highest growth rates were recorded during these seasons when salmon effluent conditioned with macroalga also had a significant beneficial effect on bivalve growth when compared to that of the control. Higher growth rates of bivalves in treatments with alga in fall and summer could due to nutrients released by the alga that the bivalves utilize as food, or to a warming effect caused by absorption of heat from sunlight by P. mollis. Results of oyster growth in winter and spring also suggest that high stocking densities of P. mollis may inhibit oyster growth.

Low growth rates of oysters in ambient sea water fall and winter can be attributed to low natural food availability, combined with low water temperatures. Results from the Winter Experiment suggest that salmon effluent or salmon effluent conditioned by addition of P. mollis does not improve oyster growth in winter because the water temperatures are too low and severely limit

growth.

Growth response of Pacific oysters and Manila clams to treatments tested during Summer, 1991 was the same. Both oysters and clams had significantly higher growth rates in the treatments with alga compared to those in salmon effluent alone or ambient seawater.

The results from the Summer Experiments, when water temperature and food availability were within the range for optimum bivalve growth, suggest that during that time of the year, there may not be an advantage in utilizing the salmon effluent because it does not result in greater bivalve growth rates than those occurring in ambient sea water.

Detailed information on optimum flow rates and stocking densities for culturing Pacific oyster should be first determined before the profitability of the polyculture system can be assessed. The successful implementation of the polyculture technique will depend on determination and manipulation of the variables that affect growth and survival of the cultured organisms, so that production can be maximized.

BIBLIOGRAPHY

Anderson, G. J., M. B. Miller and K. K . Chew. 1982. A guide to the Manila clam aquaculture in Puget Sound. Seattle: Washington Sea Grant. WSG 82-4. 45 pp.

Angell, C. L. 1986. The biology and culture of tropical oysters. I.C.L.A.R.E.M. Manila, Philippines. 42 pp.

Bardach, J. E., J. H. Ryther and W. O. McLarney. 1972. Aquaculture. New York, Wiley Interscience.

Bayes, J. C. 1981. Forced upwelling nurseries for oysters and clams using impounded water systems. Europ. Maricult. Soc., Spec. Publ. No. 7. p. 73-83.

Bayne, B. L., R. J. Thompson and J. Widdows. 1976. Physiology: I. IN: B.L. Bayne (Ed). Marine Mussels: Their Ecology and Physiology. Cambridge University Press, Cambridge. 506 pp.

Bayne, B. L. and R. C. Newell. 1983. Physiological energetics of marine mollusks. IN: A.S.M. Saleuddin and K. M. Wilbur (Ed). The Mollusca. University Press, N.Y. p. 407-515.

Bourne, N. 1982. Distribution, reproduction, and growth of the manila clam, Tapes philippinarum (Adams and Reeves), in British Columbia. J. Shellfish. Res. 2(1):47-54.

Bourne, N. 1989. Clam fisheries and culture in Canada. IN: J.J. Manzi and M. Castagna (Eds). Clam mariculture in North America. Elsevier Sc. Publ. Co. Inc. p. 357-381.

Boyle, B. J. 1989. Introduction to shellfish aquaculture in the Puget Sound region. State of Washington, Dept. of Natural Resources, Olympia, Washington. 68 pp.

Broecker, W. S. and T-H. Peng. 1982. Tracers in the sea. Columbia University, N.Y.

Brown, J. R. and E. B. Hartwick. 1988. Influences of temperature, salinity and available food upon suspended culture of the Pacific oyster, Crassostrea gigas. I. Absolute and allometric growth. Aquaculture, 70:231-251.

Chew, K. K. 1981. Proceedings of the North American oyster workshop. March 6-8, Spec. Publ. No. 1. Seattle, Wa. p. 24-25.

Chew, K. K. 1989. Manila clam biology and fishery development in western North America. IN: J.J. Manzi and M. Castagna (Eds). Clam Mariculture in North America. Elsevier Sc. Publ. Co. Inc. p. 243-261.

Crosby, M. P., R. I. E. Newell and C.J. Langdon. 1990. Bacterial mediation in the utilization of carbon and nitrogen from detrital complexes by Crassostrea virginica. Limnol. Oceanogr. 35(3):625-639.

Cushing, D. H. 1956. The seasonal variation in oceanic production as a problem in population dynamics. J. Cons. Int. Explor. Mer. 24:455-464.

Duggins, D. O., C. A. Simenstad and J. E. Estes. 1989. Magnification of secondary production by kelp detritus in coastal marine ecosystems. Science, 245:170-173.

Else, P. V. 1987. Alaska grower's manual. Mar. Advis. Bull. 17:1-5. Univ. of Alaska.

Epifanio, C. E. and C. A. Motz. 1975. Growth of oysters in a recirculating system. Proc. Natl. Shellfish. Assoc. 65:32-37.

Epifanio, C. E., R. Srna and G. Pruder. 1975. Mariculture of shellfish in controlled environments: a prognosis. Aquaculture, 5:227-241.

Foy, R. H. and R. Rossel. 1991. Loadings of nitrogen and phosphorus from a Northern Ireland fish farm. Aquaculture, 96:17-30.

Glaude, J. B. 1977. A literature review and analysis of the use of thermal effluent in the culture of the Pacific Oyster IN: ELSE, P. V., 1987. Alaska Grower's Manual. Mar. Advis. Bull 17:1-5.

Glock, J. W. and K. K. Chew. 1979. Growth, recovery, and movement of Manila clams, Venerupis japonica (Deshayes) at Squaxin Island, Washington. Proc. Natl. Shellfish. Assoc. 69:15-20

Goulletquer, M. N. and M. Heral. 1988. Energy budget of Manila clam, Ruditapes philippinarum (Adams et Reeve): production and trophic relationships in semi-closed ponds. Aquaculture, 74:331-348.

Hammen, C. S. and K. M. Wilbur. 1959. Carbon dioxide fixation in marine invertebrates. I. The main pathway in the oyster. Comp. Biochem. and Physiol. 17:289-296.

Hammen, C. S. 1966. Carbon dioxide fixation in marine invertebrates: a review. Comp. Biochem. Physiol. 17:289-296.

Hargis, W. J. and D. S. Haven. 1988. The imperilled oyster industry of Virginia. Appl. Mar. Sci. Ocean Engineer., Special Report No. 290. 130 pp.

Hartman, M., C. E. Epifanio, G. Pruder and R. Srna. 1973. Farming the artificial sea: growth of clams in a recirculating seawater system. Proc. of the Gulf and Caribbean Fisheries Institute; 26th annual session. p. 59-74.

Hepher, B. and Y. Pruginin. 1981. Commercial fish farming. Wiley- Interscience Publ. 261 pp.

Huet, M. 1986. Textbook of fish culture. Fishing New Books Ltd. Farnham, Surrey, England, 2nd Ed. 438 pp.

Jones, T. O. and G. K. Iwama. 1991. Polyculture of the Pacific oyster, Crassostrea gigas (Thunberg), with chinook salmon, Oncorhynchus tshawytscha. Aquaculture, 92:313-322.

Jorgensen, C. B. 1966. Biology of suspension feeding. Pergamon Press, 357 pp.

Kafuku T. and H. Ikenowe. 1983. Modern methods of aquaculture in Japan. Develop. Aquacult. Fish. Sci., Vol. 11. Elsevier Scientific Corp. Amsterdam. IN: ELSE, P. V. 1987. Alaska Oyster Grower's Manual Mar. Adv. Bull. 17:1-5.

Korringa, P. 1976. Farming the cupped oysters of the genus Crassostrea. Elsevier Scientific Publishing Co. New York. 224 pp.

Langton, R. W., J. E. Winter and O. A. Roels. 1977. The effect of ration size on the growth and growth efficiency of the bivalve mollusc Tapes japonica. Aquaculture, 12:283-292.

Larney, C. 1972. Aquaculture. Wiley-Interscience, New York.

Levin, J.E. 1990. Land based polyculture of marine macroalgae and Pacific salmon. M.Sc. Thesis. Oregon State University. 65 pp.

Lindsay, L. and T. Needham. 1988. Salmon and trout farming. Ellis Horwood Series in Aquaculture and Fisheries Support. Aberdeen.

Loosanoff, V. L. 1962. Effects of turbidity on some larval and adult bivalves. Proceedings of the Gulf and Caribbean Fisheries Institute. November, 1961.

Loosanoff, V. L. and H. C. Davis. 1963. Rearing of bivalve mollusks. Adv. Mar. Biol. 1:1-136.

Lucas, M. I., R. C. Newell and B. Velimirov. 1981. Heterotrophic utilization of mucilage released during fragmentation of kelp Ecklonia maxima and Laminaria pallida. II. Differential utilization of dissolved organic components from kelp mucilage. Mar. Ecol. Prog. Ser. 4:43-55.

Malouf, R. E. and W. P. Breese. 1977. Seasonal changes in the effects of temperature and water flow rate on the growth of juvenile Pacific oysters, Crassostrea gigas (Thunberg). Aquaculture, 12:1-13.

Malouf, R. E. and W. P. Breese. 1978. Intensive culture of the Pacific oyster Crassostrea gigas (Thunberg), in heated effluents. Publ. no. ORESU-T-78-003 of Oregon State University Sea Grant College Program. 41 pp.

Manahan, D. T. and D. J. Crisp. 1982. The role of dissolved organic material in the nutrition of pelagic larvae: amino acid uptake by bivalve veligers. Amer. Zool. 22:635-646.

Manahan, D. T. 1989. Amino acid fluxes to and from seawater in axenic veliger larvae of a bivalve Crassostrea gigas. Mar. Ecol. Prog. Ser. 53:247-255.

Mann, K. H. 1972. Ecological energetics of the sea-weed zone in a marine bay on the Atlantic coast of Canada. II. Productivity of the seaweeds. Mar. Biol. 14:199-209.

Mann, K. H. 1973. Seaweeds: their productivity and strategy for growth. Science, 182:975-981.

Mann, R. 1979. The effect of temperature on growth, physiology, and gametogenesis in the Manila clam <u>Tapes philippinarum</u>. J. Exp. Mar. Biol. Ecol. 38:121-133.

Mann, R. and J. H. Ryther. 1977. Growth of six species of bivalve mollusks in a waste recycling-aquaculture system. Aquaculture, 1:231-245.

Manzi, J. J., N. H. Hadley and M. B. Maddox. 1986. Seed clam, <u>Mercenaria</u>, culture in an experimental-scale upflow nursery system. Aquaculture, 54:301-311.

Matthiessen, G. C. 1971. A review of oyster culture and the oyster industry in north America. Contribution 2528 from the Woods Hole Oceanographic Institution 51 pp.

McNeil, W. 1988. Mariculture: and aid or hindrance to management?. Trans. 53rd N.A. Wild. & Nat. Res. Conf. p. 569-573.

Newell, R. C. and M. I. Lucas. 1981. The quantitative significance of dissolved and particulate organic matter released during fragmentation of kelp in coastal waters. Kieler Meeresforsch., Sonderh. 5:56-369.

Newell, R. C. and J. G. Field. 1983. Relative flux of carbon and nitrogen in a kelp dominated system. Mar. Biol. Letters 4:249-257.

National Oceanic and Atmospheric Administration, 1990-1991. Database from the National Marine Fisheries Service, Pacific Fisheries Environmental Group.

Organization for Economic Co-operation and Development. 1989. Aquaculture. Developing a new Industry. Paris, France. p. 7-15.

Quast, W. D., M. A. Johns, D. E. Pitts, Jr., G. A. Matlock and J. E. Clarck. 1988. Texas oyster fishery management plan. No. 1., 44 pp.

Quayle, D. 1969. Pacific oyster culture in British Columbia. Fish. Res. Bd. Can. 169:192.

Rice, M. A. and G. C. Stephens. 1987. Uptake and internal distribution of exogenously supplied amino acids in the Pacific oyster, <u>Crassostrea gigas</u> (Thunberg). Aquaculture, 66:19-31.

Ricker, W. E. 1975. Computation and interpretation of biological statistics of fish populations. Can. Fish. Res. Bd. Bull. No. 191.

Rodhouse, P. G. and M. O'Kelly. 1981. Flow requirements of the oysters Ostrea edulis L. and Crassostrea gigas Thunb. in an upwelling column system of culture. Aquaculture, 22:1-10.

Ryther, J. G. and C. S. Yentsch. 1957. The estimation of phytoplankton production in the ocean from chlorophyll and light data. Limnol. Oceangr. 2:281-286.

Ryther, J. H. and J. E. Bardach. 1968. The status and potential to Aquaculture. Amer. Inst. of Biol. Sci., Springfield, Virginia.

Ryther, J. H., J. C. Goldman, C. E. Gifford, J. E. Huguenin, A. S. Wing, J. P. Clarner, L. D. Williams and B. E. LaPointe. 1975. Physical models of integrated waste recycling-marine polyculture systems. Aquaculture, 5:163-177.

Schaffer, D. 1989. Statistical methods for research workers. Statistics 451, Class Notes and Lab. Manual, Dept. of Statistics, Oregon State University.

Shumway, S. E., T. L. Cucci, R. C. Newell and C. L. Yentsch. 1985. Particle selection, ingestion and absorption in filter feeding bivalves J. Exp. Mar. Biol. Ecol. 91:77-92.

Snedecor, G. W. and W. G. Cochran. 1980. Statistical methods. Univ. Press. Ames, Iowa. 7th Ed.

Sokal, R. R. and F. J. Rohlf. 1969. Biometry, the principles and practice of statistics in biological research. W.H. Freeman and Company. 776 pp.

Spencer, B. E. and B. T. Hepper. 1981. Tide-powered upwelling systems for growing nursery-size bivalves in the sea. Europ. Maricult. Soc., Spec. Publ. No. 7. p. 283-309.

Spencer, B. E., M. J. Akester and I. Mayer. 1986. Growth and survival of seed oysters in outdoor pumped upwelling systems supplied ewith fertilized sea water. Aquaculture, 55:173-189.

Stephens, G. C. 1967. Dissolved organic material as a nutritional source for marine and estuarine invertebrates. IN: Estuaries. G.H. Lauff(Ed). Publ. No. 83, Amer. Assoc. Adv. Sci. p. 367-373.

Strickland, J. D. H. and T. R. Parsons. 1984. A practical handbook of seawater analysis Bull. 167 Fish. Res. Bd. Can.

Tenore, K. R., J. C. Goldman and J. P. Clarner. 1973. The food chain dynamics of the oyster, clam, and mussel in an aquaculture food chain. J. Exp. Mar. Biol. Ecol. 12:157-165.

Utting, S. D. 1988. The growth and survival of hatchery-reared Ostrea edulis L. spat in relation to environmental conditions at the on-growing site. Aquaculture, 69:27-38.

Urban, E. R. and C. J. Langdon. 1984. Reduction in costs of diets for the american oyster, Crassostrea virginica (Gmelin), by the use of non-algal supplements. Aquaculture, 38:277-291.

Walne, P. R. 1972. The influence of current speed, body size and water temperature on the filtration rate of five species of bivalves. Mar. Biol. Ass. U.K. 52:345-374.

Walne, P. R. and B. E. Spencer. 1974. Experiments on the growth and food conversion efficiency of the spat of Ostrea edulis L. in a recirculation system. J. Cons. Int. Explor. Mer. 35(3):303-318.

Williams, J. G. 1980 b. Growth and survival in newly settled spat of the Manila clam, Tapes japonica. Fish. Bull. 77(4):891-900.

Williams, P. 1981. Offshore nursery-culture using the upwelling principle. Europ. Maricult. Soc., Spec. Publ. No. 7. p. 311-315.

Winter, J. E. 1978. A review on the knowledge of suspension feeding in lamellibranchiate bivalves, with special reference to artificial aquaculture systems. Aquaculture, 13:1-33.

Wright, R. T., R. B. Coffin, C. P. Ersing and D. Pearson. 1982. Field and laboratory measurements of bivalve filtration of natural marine bacterioplankton. Limnol. Oceanogr. 27(1):91-98.

Wright, S. H. 1982. A nutritional role for amino acid transport in filter-feeding marine invertebrates. Amer. Zool. 22:621-634.

Wright, S. H. 1988. Amino acid transport in the gill epithelium of a marine bivalve. Comp Biochem. Physiol. 90A(4):635-641.

APENDIX

APPENDIX I

Results on two-way analyses of variance (ANOVA) of mean water temperature for Summer Experiment #1 (June 3 - July 4, 1991). Legend: F+A= salmon effluent conditioned by 18 g/l <u>Palmaria</u> <u>mollis</u>; F-A= salmon effluent alone; R+A= ambient seawater conditioned by 18 g/l <u>P</u>. <u>mollis</u>, R-A= ambient seawater alone (control).

<u>Summer Experiment #1</u>

<u>Source of var</u>.	<u>Sum of squares</u>	<u>d.f</u>	<u>Mean square</u>	<u>F-ratio</u>	Sig. <u>level</u>
Main effects	27.691333	2	13.845667	18.055	0.0000
Fish	1.290667	1	0.290667	1.683	0.1998
Alga	26.400667	1	26.400667	34.427	0.0000
2-Factor Interac.	0.7706667	1	0.7706667	1.005	0.3204
Fish Alga	0.7706667	1	0.7706667	1.005	0.3204
Residual	42.944000	56	0.7668571		
Total	71.606000	59			

Results on two-way analyses of variance (ANOVA) of mean water temperature for Summer Experiment #2 (July 19-Aug 17, 1991). Legend: F+A= salmon effluent conditioned by 18 g/l Palmaria mollis; F-A= salmon effluent alone; R+A= ambient seawater conditioned by 18 g/l P. mollis and R-A= ambient seawater alone (control).

Summer Experiment #2

Source of var.	Sum of squares	d.f	Mean square	F-ratio	Sig level
Main effects	67.216667	2	33.608333	74.194	0.0000
Fish	1.066667	1	0.066667	2.355	0.1305
Alga	66.150000	1	66.150000	146.034	0.0000
2-Factor Interac	0.600000	1	0.600000	1.325	0.2547
Fish Alga	0.600000	1	0.600000	1.325	0.2547
Residual	25.366667	56	0.4529762		
Total	93.183333				