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Crassostrea gigas (Thunberg), IN HEATED EFFLUENTS

Robert E. Malouf
and
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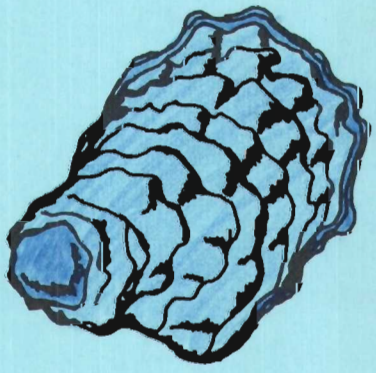

OREGON STATE UNIVERSITY
SEA GRANT COLLEGE PROGRAM
Publication no. ORESU-T-78-003

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related publications

HATCHERY PRODUCED PACIFIC OYSTER SEED; ECONOMIC FEASIBILITY ON CULTCH IN THE PACIFIC NORTHWEST. Kwang K. Im and R. Donald Langmo. 1977. 80 pp. $2.50 (ORESU-T-77-010).

Investigates the economic feasibility of producing hatchery seed in the Pacific Northwest, taking into account economic, technical and biological factors; develops costs for five different levels of output that are currently within practical commercial capacities; and establishes cost at each level of production for five methods of cultch preparation.

RESIDUAL TOXICITY OF OZONIZED SEAWATER TO OYSTER LARVAE. J.M. DeManche; et al. 1975. 7pp. (ORESU-T-75-003).

Presents preliminary findings on the toxicity of ozonized seawater to oyster larvae and subsequent detoxification procedures.

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contents

introduction

Aquaculture can potentially produce very high food yields from relatively small areas in which ponds or raceways provide a controlled environment. However, realizing this potentially high production requires that the aquaculture facility provide conditions that are as close to optimum as possible for the animal being cultured. Providing optimum conditions in turn requires that the aquaculturist have control over the culture system's environment. Water temperature is one of the most critical environmental parameters for the growth and well-being of marine animals, but the cost of controlling temperatures in large volumes of flowing seawater is prohibitive. Coastal nuclear or fossil fuel electrical generating plants use seawater as a coolant and produce large quantities of warmed water as a waste by-product. The availability of this otherwise wasted heat energy may make control of water temperatures in a large-scale commercial aquaculture facility technically and economically feasible.

At Oregon State University, we began research in 1971 on the potential beneficial uses of heated effluents in aquaculture. During the course of the study, we conducted experiments to assess the feasibility of culturing the Pacific oyster (*Crassostrea gigas*) and several species of salmon in warmed seawater. The work on salmon culture, completed in 1974, is described in a number of publications. Our objectives in this report are to discuss the results of our work with the Pacific oyster and to consider the feasibility of using heated effluents to culture this species intensively.

Although our conclusions are based primarily on the results of our own research, we also draw upon other researchers' studies to reinforce our findings. Unfortunately, must of the previous work on intensive oyster culture and on the effects of elevated temperatures on oysters has dealt with species other than *C. gigas*. Such work can indicate the results that we could expect from *C. gigas* under similar conditions, but we caution the reader to

keep in mind that there are very real physiological differences among the species of oysters. Regardless of the species involved, the geographic location of the various studies must also be considered in evaluating their applicability to our specific area of interest. Natural environmental conditions interact with the effects of elevated temperatures and with the artificial conditions created by the culture system to alter the mollusks' response. Our data provides some specific examples of this problem.

DEFINITION OF TERMS

Our studies were primarily concerned with intensive oyster culture. Intensive culture means systems of holding and rearing animals in a pond, raceway, or other enclosure with controlled water exchange and distribution; this implies that the number and type of macroorganisms contained in the system can also be controlled. An intensive culture system contains a greater than natural density of the cultured animal. The degree of intensivy depends on the completeness of control and on culture density; for example, intensive oyster culture ranges from enclosures separated from the sea by dikes having tidal flood gates to entirely closed recirculating culture systems. Between these two extremes, a number of experimental and commercial culture systems have varying degrees of control over the animals' environment. Some of these systems will be considered in greater detail in later sections.

The potential exists for using heated effluents in other aspects of oyster aquaculture. Heated effluents could provide the relatively high water temperatures required for production of oyster "seed"--post larvae, preplanting--in a hatchery. Oysters could also be grown out, using more traditional methods, in the thermal plume from a power plant. These alternative uses for heated effluents in oyster culture will be considered briefly.

Finally, "feasibility" in this study connotes probability of biological success; that is, we discuss the growth and survival of oysters under various environmental conditions, and draw some conclusions about heated effluents' potential effects on oysters in an intensive culture situation. However, economic and technical feasibility, which are beyond the scope of this project, must also be considered before general recommendations concerning commercial feasibility can be made.

ADVANTAGES OF INTENSIVE OYSTER CULTURE

Pacific oysters have been cultivated in Oregon waters since the mid 1930s. Traditionally, these oysters were produced from seed, attached to old oyster shell "cultch" --substrate for attachment--was either spread in patterns or somewhat randomly broadcast onto estaurine mud flats to grow to maturity. Harvesting of the market-sized oysters was accomplished with oyster tongs or dredges, or simply by hand harvesting at low tide. This culture method requires relatively little capital investment, but it also suffers from a number of significant disadvantages. For example, the traditional culture method requires the use of oyster seed attached to cultch. However, this type of seed is becoming expensive and difficult to obtain at any price.

Commercial oyster hatcheries prefer to produce the so-called "cultchless" oyster seed. Cultchless seed consists of individual oysters generally 3-10 mm in length and not attached to any substrate. This type of seed is easy to produce and can be shipped anywhere in the world without special handling. One million cultchless oyster seed can very easily be contained in a 2-cubic-foot insulated shipping container. An equal number of standard cultch seed would require shipping over 150 bushels of oyster shell. Cultchless oyster seed's advantage has seriously limited hatchery production of cultched seed and, in turn, has further reduced the availability of seed for traditional oyster culture methods in Oregon.

Cultchless oyster seed requires more care and handling than seed attached to shells or other cultch materials. Its growing use in Oregon's estuaries has brought fundamental changes to the industry. Specifically, cultchless seed has forced the oyster industry to increase the intensity of oyster culture in Oregon. At present, cultchless seed is cultured in trays suspended from rafts in bays, but raceway or pond culture methods will probably soon become a reailty.

In addition to satisfying the requirement for careful handling of cultchless seed, intensive culture offers some distinct advantages over traditional culture methods. Raceway or pond culture provides protection for the growing crop of highly vulnerable young oysters. Predators such as crabs and fish can be excluded from the oysters' environment, and damage to the culture

facility from storms can be avoided. Water of very low salinity occasionally occurs in the oyster rearing areas of some of Oregon's estuaries following periods of heavy rains. Mature oysters, able to close their shells, can avoid the harmful effects of the reduced salinity for hours or even days at a time. Small oysters, on the other hand, are vulnerable to the effects of such freshets, and may suffer heavy mortalities. An intensive culture operation using a pond or raceway could isolate its water supply from the bay by turning off pumps or by closing tide gates. In this manner, oyster growers could exclude water of dangerously low salinity from the oysters' environment.

Other advantages to intensive oyster culture include ease of access and harvest, and increased protection of the oyster growing area. Currently, in Oregon, oyster grounds are leased from the state, not owned by the oyster grower, although the grower does have some of the rights of ownership. Moreover, these oyster grounds, as public property, are subject to control and regulation by a number of different agencies, including the U.S. Army Corps of Engineers, the Oregon Department of Environmental Quality, the U.S. Coast Guard, and others. Intensive culture, removed from navigable waterways, would still be subject to water pollution regulation, but would avoid many of the restrictions placed on traditional culture grounds.

Intensive oyster culture offers the possibility of increasing seawater's natural ability to support oyster growth. This might be accomplished through the use of fertilizers to increase the density of the oysters' naturally occurring algal food, or through the use of some yet to be developed artificial food for oysters. We must recognize that in the absence of enhancement or supplementation of oysters' natural food, intensive culture would not increase the estuary's overall productivity. Intensive oyster culture directs the sea's natural productivity into an edible and marketable product more efficiently than traditional culture methods, but, perhaps more important, it provides the oyster farmer with the opportunity of increasing or supplementing that natural productivity.

Generally, intensive culture practices afford the aquaculturist increased control over the environment in which the crop is grown, providing at least the opportunity to control water temperature, water flow rate, food density, and salinity. This control over the environment in turn affords the chance to optimize conditions for the growth and survival of the oyster crop in much the same way that conditions are optimized for the growth and survival of terrestrial livestock.

INTENSIVE CULTURE BACKGROUND

Oysters have been cultured for several centuries and a review of some of the important aspects involving the growth of oysters under controlled conditions seems appropriate as a logical first step in evaluating the biological feasibility of intensively culturing the Pacific oyster in heated effluents.

The French have cultured the European oyster, *Ostrea edulis*, in shallow ponds or *claires* for hundreds of years. Recently they have begun to use *claires* for the Portugese oyster, *Crassostrea angulata*. However, although the French cultivate these ponds very intensively and many even use fertilizers to increase algae production, the *claires* are not generally used to grow oysters from spat to market size, but their continued success indicates that oysters can be maintained in and will thrive under such conditions.

On the East Coast of the United States a great deal of work has been done with the Eastern or American oyster, *Crassostrea virginica*, a close relative of *Crassostrea gigas*. In 1904, Everman reported that adding fertilizer to ponds containing oysters created a dense bloom of algae in the ponds. However, he found that the oysters acquired a "marshy" flavor from the "undesirable" species of algae in the pond. Nevertheless, growth improved in the pond-reared oysters, demonstrating over 70 years ago that oysters could be cultured intensively under controlled conditions.

In 1959, Carriker reported the results of detailed studies on the feasibility of culturing *C. virginica* in ponds constructed on Gardiner's Island in Long Island Sound. He found that ponds permanently isolated from seawater exchange would not support the growth of oysters, but that if an adequate source of food-bearing water could be maintained the culture techniques showed promise. He also pointed out that at that time very little information was available to permit definition of what constitutes an "adequate exchange of food-bearing water." Interestingly, Carriker suggested 20 years ago that a program of selective breeding was needed to develop a strain of oysters adapted to intensive culture practices.

The U.S. Fish and Wildlife Service supported studies in the early 1960s to evaluate the potential of pond culture for growing oysters in the Chesapeake Bay region. These studies were carried out by Shaw (1964, 1968), and were conducted in four .1 hectare (one-fourth acre) ponds constructed at the Bureau of Commercial Fisheries headquarters in Oxford, Maryland. The ponds were supplied with water pumped directly from Chesapeake Bay. Shaw found that the condition of oysters placed in these ponds declined steadily owing to lack of food. The study's official conclusion was that artificial ponds appear to have commercial potential for oyster culture, but commercial application would require refinement of techniques and development of methods to increase the oysters' food supply.

In 1970, Ryther began research on the feasibility of using treated sewage as a source of fertilizer for culturing large quantities of planktonic marine algae. Ryther and his colleagues at the Woods Hole Oceanographic Institution proposed using algae produced in this manner to feed oysters or other mollusk in raceway culture systems. In a series of experiments with *C. virginica* and other bivalves, Ryther found that careful use of sewage increased the density of algae in seawater, and that this algae could be used to grow oysters.

However, the success of the system depended on the ability of the oyster farmer to control temperatures. Without temperature control, the algal species composition in the ponds could not be maintained, and consequently, species of algae that were poor food for oysters occasionally dominated the cultures. More importantly, lack of temperature control during the winter months caused the water temperatures in the rearing ponds to drop well below optimum temperatures for oyster growth. Therefore, during the cold months, the mollusks' growth was minimal regardless of the food available to them. Ryther's important and imaginative pilot studies demonstrated that intensive oyster culture is feasible, but its success depends on control of both temperature and food supply.

In 1972, researchers at the University of Delaware began construction of one of the most intensive pilot oyster culture systems in the world. The system is entirely closed: seawater is recycled through a water treatment plant to remove uneaten food and waste products. Food for the oysters (*C. virginica*) is produced in large-scale, single-species algal cultures, and is metered into the oyster rearing tanks in controlled amounts. Water temperature and flow rates are also controlled.

After five years of research, Epifanio and his University of Delawave colleagues have shown that oysters can be grown under completely controlled conditions. However, their work also underlines how little we known about many of the nutritional and environmental requirements of oysters. For example, there were very distinct differences in the growth rates of different groups of oysters fed different species of cultured algae, but there is at present no adequate explanation of the nutritional benefit to oysters of the different algal species.

Experiments conducted over a number of years have provided at least a partial definition of the conditions under which oysters can be intensively cultured. These studies have also shown that the concept of intensive oyster culture is biologically sound: oysters can be grown under controlled conditions. However, we do not yet know for certain what environmental conditions are optimal for oyster growth and further research is necessary before we can understand how environmental factors interact with each other and with food quantity and quality to affect the growth of oysters in intensive culture.

assessment of feasibility

To assess the biological feasibility of intensively culturing the Pacific oyster, we conducted a series of experiments over a period of five years. These experiments were carried out at Oregon State University's Marine Science Center in Newport and at the Marine Fisheries Laboratory in Port Orford.

Two basic types of experiments were conducted. The first involved determination of rates of growth and survival of Pacific oysters at various combinations of temperatures and water flow rates. These experiments were repeated seasonally to monitor changes in natural food availability. The second type of experiment was conducted in a recirculating seawater system without natural food. Also conducted at various temperatures, these experiments permitted definition of an energy budget for juvenile oysters. Secondarily, the "closed system" experiments investigated the possibility of using cultured algae to supplement oysters' natural food. These two types of experiments enabled us to evaluate the feasibility of intensive oyster culture in power plant effluents.

EXPERIMENTS USING UNFILTERED SEAWATER

During the course of this study, a number of different types of experiments were conducted. To avoid confusion concerning the objectives, design, and results of these experiments, we will discuss each type separately after a brief statement of methodology that applies to all of the experiments. The experiments were conducted to: (1) determine the feasibility of culturing Pacific oysters in seawater taken from an open coastal location; (2) determine the relationship between water temperature and oyster growth in natural water; (3) monitor seasonal fluctuations in the natural food of oysters and to relate those fluctuations to changes in oyster growth rate; and (4) determine the relationship between oyster growth rate and water flow rate at various temperatures.

General Methods

All of the Newport and Port Orford experiments using unfiltered seawater used the same basic type of culture system. The system consisted of a series of head tanks, one for each temperature included in the design of the experiment. Each head tank received a flow of unfiltered seawater at ambient temperature. Vycor immersion heaters controlled by thermoregulators were placed in all but one of the head tanks to heat the water to the desired temperatures. Heated water was aerated to prevent supersaturation with atmospheric gases because of the increase in temperature. Water supersaturated with atmospheric gases causes gas-bubble disease in many marine organisms, including oysters (Malouf et al. 1972).

Water of appropriate temperatures flowed through glass tubing that was bent and inserted through rubber stoppers. The water flow rate was controlled by rotating the glass bend in the stoppers. The systems maintained water flow rates that were generally within $\pm 1^{o}C$ of the reported temperature.

During experiments, oysters were placed on plastic screens in shallow plastic pans through which water from one of the head tanks flowed. The pans were placed in water baths that received a flow of excess water from the head tanks, which served to dampen possible fluctuations in water temperature within the pans.

Measurements of oyster growth were based on changes in shell length or meat weight. Shell length, for purposes of this study, is defined as the maximum dimension from the umbo to the ventral margin of the shell. Technically this dimension is shell height, but it is also the shell's greatest dimension and is most commonly referred to as length. Growth in terms of shell length was determined by periodic measurement of randomly selected samples of oysters from each treatment group. Large animals were measured to the nearest millimeter with calipers. Small animals, used in only one experiment, were measured to the nearest .1 millimeter with a micrometer eyepiece on a dissecting microscope.

To provide a measurement of the initial weight of the oysters' meat, a random sample was drawn from the pooled group prior to the start of the experiment. The wet or ash-free dry weight of the meats of individual oysters or groups of oysters from the sample were determined using standard procedures (Malouf and Breese 1977).

Relative growth rates were calculated from meat weights or shell lengths from the equation:

$$k = \frac{\ln W_2 - \ln W_1}{(T_2 - T_1)}$$

where:

W_1 = the initial mean weight or shell length

W_2 = the final mean weight or shell length

and $(T_2 - T_1)$ = the duration of the experiment in days

The coefficient, k, multiplied by 100 yields percent change per day (Warren 1971).

Cultchless oysters used in the experiments were obtained from commercial oyster hatcheries in Washington and California. Oysters attached to shell cultch, used in only one experiment, were produced by the pilot oyster hatchery at Oregon State University's Marine Science Center in Newport. In all cases, the oysters were held in flowing seawater at ambient temperatures for at least two weeks prior to the start of an experiment. At the beginning of an experiment, randomly selected oysters were placed directly into seawater at the various treatment temperatures. The animals were not acclimated to temperature.

Growth and Survival of Pacific Oysters at an Open Coastal Location

Public attitudes and current coastal land use planning make it unlikely that future power generating plants will be constructed on any of Oregon's limited estuarine waters. Therefore we must establish whether or not Pacific oysters can survive and grow in seawater pumped from the ocean at an open coastal site before we consider the feasibility of using heated power plant effluents to culture this species. For this purpose, paired systems were constructed at the Marine Science Center in Newport, an estuarine location, and at Port Orford, an open coastal site. Experiments were then conducted at the two locations to monitor the growth and survival of juvenile Pacific oysters provided with equivalent water flow rates and temperatures.

The first experiment involved the use of cultchless juvenile Pacific oysters. Fifty animals were placed in each of four plastic trays receiving 400 ml/min of seawater at one of four temperatures, 10°, 15°, 18°, and 21°C. The oysters were held under these conditions for 40 days (January 16 to March 12, 1973). Their growth and survival over this period was monitored in replicate systems at the two locations.

Results show that growth rate was low at both locations (Table 1). Mortality was somewhat higher in the Port Orford system, but was directly related to temperature at both locations. On the basis of this experiment, no definitive statements are possible concerning the relative suitability of the two locations for an oyster culture facility. The data indicate that poor growth occurs during the late winter months, and that increased temperatures during this period can result in high mortality rates. This seasonal aspect of oyster growth and survival was investigated in a later experiment.

The second, longer term experiment in this series was identical to the first, but was conducted later in the spring (March 30-June 22, 1973) and lasted 83 days. The results of this experiment are very similar to those of the first (Table 2). Growth and survival rates were greater for animals held in Newport. Mortality rates among the Port Orford oysters were clearly higher at the increased temperatures. This indicated that food was probably more plentiful at the Newport site. Stress from the combination of high temperature and low food density caused increased mortality among the oysters held on the open coast.

The third short-term experiment compared growth and survival in the two locations using small spat attached to shell cultch. Two hundred spat were selected for relatively uniform sizes and even distribution on the shell cultch. All other spat on the shells were removed. Shell pieces holding a total of 50 spat were placed in each of four trays receiving 400 ml/min of sea water at the required temperature. Weekly measurements of shell length were made for four consecutive weeks (May 27 to June 24, 1973). Systems and procedures as described above were replicated at the two sites.

Unlike the previously described experiments, this experiment showed good survival and improved growth among the oysters held in Port Orford. At all but the ambient seawater temperature (10°C), the oysters actually grew better at Port Orford than in the estuarine waters at Newport.

Obviously this was a short-term experiment, involving only one season of the year. However, the results show that there is nothing inherently harmful to Pacific oysters in full seawater, and that during some periods of the year the natural food of oysters is more plentiful in the estuary than on the open coast. This difference in food availability is reflected in the animals' growth and survival. However, when food is available, good growth of young Pacific oysters can be obtained at an open coastal site.

Our observations, supported by other studies not necessarily intended to compare coastal and estuarine waters, show that *Crassostrea gigas* can survive and grow well in full-strength seawater if sufficient food is available. In one such study, conducted at the Port Orford laboratory, juvenile Pacific oysters grew from 8 mm to approximately 55 mm in length in a period of nine months.

Askew (1972) concluded from studies in England that "lower salinities are necessary for successful breeding of *C. gigas*, but for the purpose of growing and fattening seed oysters, the higher salinities appear to be no disadvantage." Askew based his statement on the results of an experiment in which he cultured *C. gigas* spat in estuarine and oceanic locations in England. The average monthly instantaneous growth rate for the estuarine site was 0.233, while growth rates at the oceanic site averaged 0.241.

Relationship Between Temperature and Growth of Pacific Oysters in the Absence of Supplemental Feeding

The seven experiments conducted during this phase of the study were carried out at Oregon State University's Marine Science Center in Newport. Systems and procedures were as described in "General Methods." The objectives of the experiments were to define the relationship between water temperature and the growth of juvenile Pacific oysters, and to determine how that relationship varies with season.

The results of the seven experiments showed that, despite extreme seasonal fluctuations in the absolute values obtained for growth rate, the general relationship between temperature and oyster

	Temp °C	Initial Length(mm)	Final Length(mm)	Growth Rate (% per day)	% Mortality
Newport	10	26.4	28.7	0.21	9
	15	24.8	27.1	0.22	35
	18	26.6	28.3	0.15	38
	21	25.8	27.7	0.18	49
Port Orford	10	24.2	25.0	0.08	28
	15	25.6	25.2	0.00	38
	18	23.4	23.8	0.04	46
	21	26.7	26.4	0.00	58

Table 1. Comparison of shell growth and mortality rates among juvenile Pacific oysters reared at an estuarine location (Newport) and at an open coastal site (Port Orford). Fifty animals per treatment received a water flow of 400 ml/min. The experiment was conducted from January 23 to March 18, 1973.

	Temp °C	Initial Length(mm)	Final Length(mm)	Growth Rate (% per day)	% Mortality
Newport	10	26.4	32.0	0.23	13
	15	26.1	32.5	0.26	14
	18	25.1	31.1	0.26	14
	21	25.6	30.1	0.20	14
Port Orford	10	27.3	28.6	0.06	16
	15	25.8	29.4	0.16	40
	18	25.4	26.2	0.04	46
	21	25.7	25.8	0.00	66

Table 2. Comparison of shell growth and mortality rates among juvenile oysters reared at an estuarine location (Newport) and an open coastal location (Port Orford). Fifty animals per treatment were provided with 400 ml/min of unfiltered sea water. The experiment was conducted from March 30 to June 22, 1973.

	Temp °C	Initial Length(mm)	Final Length(mm)	Growth Rate (% per day)	% Mortality
Newport	10	3.7	5.7	1.40	2
	15	3.7	5.9	1.51	5
	18	3.9	6.7	1.75	0
	21	3.7	5.8	1.45	2
Port Orford	10	4.7	6.6	1.10	6
	15	4.1	9.3	2.64	10
	18	4.1	10.3	2.97	2
	21	4.1	10.5	3.00	0

Table 3. A comparison between shell growth of spat of the Pacific oyster attached to shell clutch held open coastal location (Port Orford) and an estuarine location (Newport). Experiment conducted from May 27 to June 24, 1973. Water flow rate at both sides was 400 m./min per 100 spat. Values given are means of 50 measurements per treatment.

growth remained suprisingly constant (Table 4). The nature of this relationship is apparent in Fig. 1 and 2, which show the mean growth rates for all water flows at a given temperature, in terms of shell growth and meat growth respectively. These data show little or no growth advantage for water temperatures in excess of 15°C. Significantly, mortality rates were always higher at elevated temperatures (Table 4).

Seasonal Fluctuations in Natural Food and Oysters Growth Rate

Five of the seven experiments described in the preceding section permit comparison of the growth of Pacific oysters at various times of the year. The combination of 8ml per oyster per minute of water flow at a temperature of 15°C, with animals having an initial shell length of 20-25mm, was included in four of the experiments (numbers I, II, III, and V). A fifth experiment (number IV) included water flow rates that bracketed 8ml per oyster per minute, so that graphical interpolation could be used to estimate growth at 8ml per oyster per minute. These five experiments allowed measurement of seasonal fluctuations in oyster growth rate, in the absence of fluctuations in temperature or changes in the size of the animals.

Available evidence indicates that oysters rely primarily on particulate organic material as food, although they can apparently absorb and utilize certain dissovled materials. There is no evidence that this particulate matter must be living. Therefore, we determined that measurement of particulate organic carbon (POC) and nitrogen provided us with the best indication of the natural food of oysters.

Weekly sample for POC and nitrogen in seawater were drawn from the head tanks used in the oyster growth experiments. Sampling continued throughout the year even when oyster growth experiments were not in progress. A 250 ml sample from a head tank (at ambient temperature) was divided into two 100 ml samples for analysis. The 100 ml samples were gently filtered through Whatman

Expt. No.	Date Started-Ended	Flow Rate (ml/oyster/min)	Initial Size Length (mm) - Weight (mg)		Temp. °C	Shell Growth (k x 100)	Rate Growth (k x 100)	Percent Mortality
I	Jan. 23-March 18	8	25.8	-	10	0.16	-	9.3
"	"	"	"	-	15	0.16	-	35.3
"	"	"	"	-	18	0.12	-	38.0
"	"	"	"	-	21	0.14	-	48.7
II	March 30-June 22	8	25.8	-	10	0.23	-	13.0
"	"	"	"	-	15	0.26	-	14.0
"	"	"	"	-	18	0.26	-	14.0
"	"	"	"	-	21	0.19	-	14.0
V	Aug. 7-Sept. 9	8	22.7	12.1	11	0.63	+1.99	-
"	"	"	"	"	15	0.81	+1.72	-
"	"	"	"	"	19	0.34	+1.28	-
"	"	"	"	"	23	0.00	-0.12	-
VI	Sept. 28-No. 14	2	-	2.78	11	-	+0.16	2.0
"	"	"	-	"	15	-	+0.07	7.0
"	"	"	-	"	19	-	-0.73	17.0
"	"	"	-	"	23	-	-1.72	29.0
VII	March 23-May 17	0.66	3.17	0.102	11	0.32	+1.28	-
"	"	"	"	"	15	0.92	+2.54	-
"	"	"	"	"	19	0.41	+0.90	-
"	"	"	"	"	23	0.32	+0.71	-

Table 4. Results of five experiments conducted to determine the effects of temperature on the growth of shell and meat in juvenile Pacific oysters during different times of the year. Flow and temperature were controlled; the sea water was otherwise untreated.

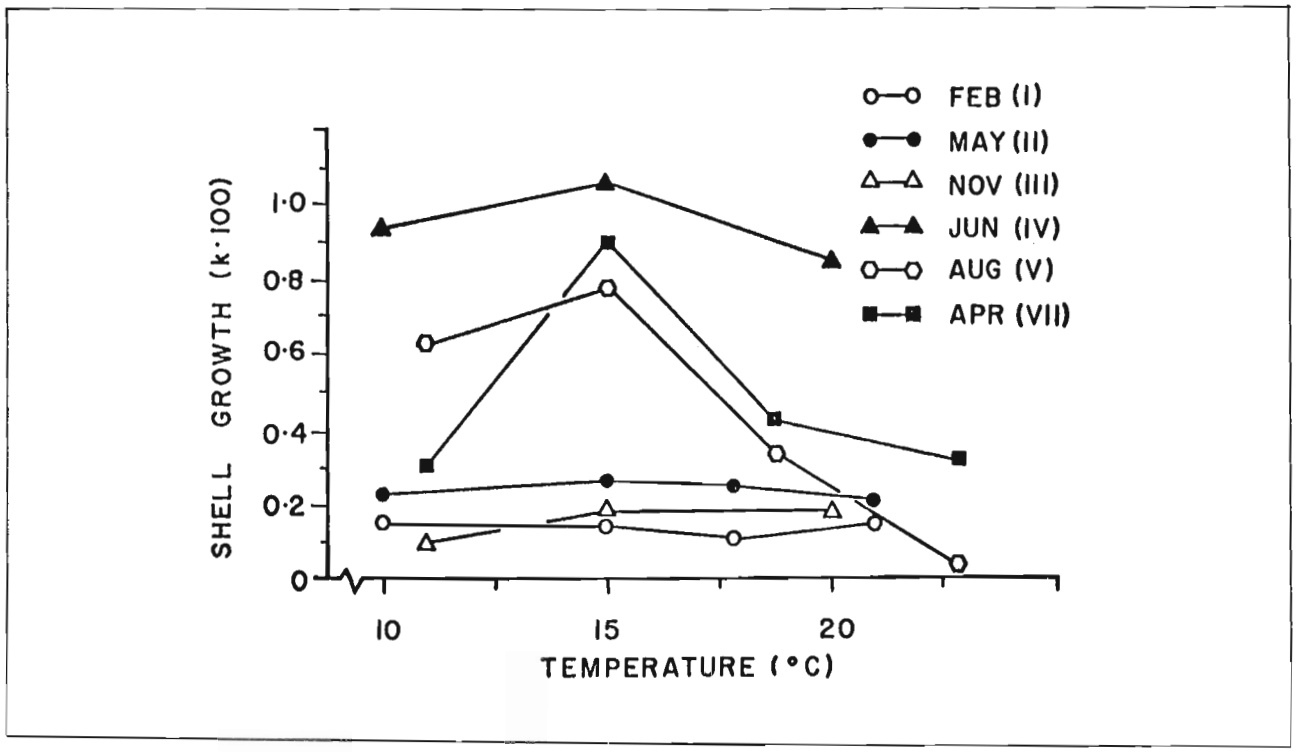

Fig. 1. The observed relationship between water temperature and shell growth of juvenile Pacific oysters, *Crassostrea gigas*. Data are from six experiments conducted at different times of the year as shown.

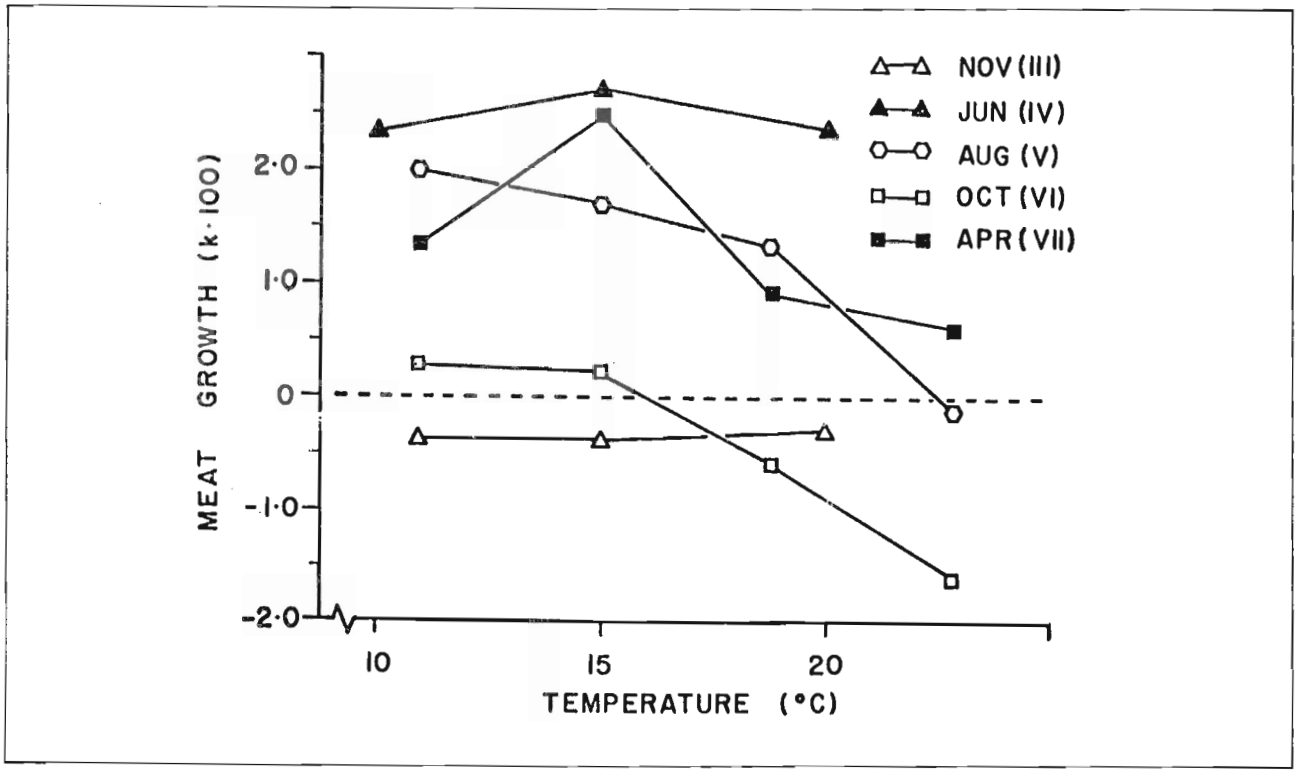

Fig. 2. The observed relationship between water temperature and meat growth of juvenile Pacific oysters, *C. gigas*. Data are from five experiments conducted at different times of the year.

GF/A glass fiber filter papers cut to fit in a 13 mm Millipore Swinnex filter holder. The procedures followed were developed by Donagjay and Small (1977) to minimize the loss of organic material during filtration. Particulate organic carbon and nitrogen were determined using a Carlo-Erba carbon-hydrogen-nitrogen-oxygen analyzer.

The results of the five growth experiments are shown in Table 5. The extreme fluctuations in oyster growth is apparent (Fig. 3 and 4). Since experiments used sexually immature animals at constant temperature, observed changes in growth were probably a result of changes in available food for the oysters. The close relationship between the oyster growth rates and the density of particulate organics (particularly nitrogen) observed in the incoming seawater supports this conclusion (Fig. 3 and 4).

It is also unlikely that the observed fluctuations were caused by changes in the salinity of the estuarine water. Though periods of little or no growth correspond to reduced salinity during the winter months, a similar cessation of growth was also observed among oysters held at Port Orford, a location that did not show significant fluctuations in salinity.

These data indicate that very little growth can be expected in juvenile oysters between the months of September and April. Food supply during warm months appears sufficient to support rapid growth, but clearly some supplemental feeding is necessary to take full advantage of artificially elevated temperatures during the winter.

Although the reported particulate organic carbon and nitrogen values may relate to available food, the measurements were not intended to reflect more than relative conditions in the bay. The ranges of particulate organics reflect values that might be expected at an estuarine location, but can not be used as a rigorous definition of the carbon or nitrogen requirements of the animals. We believe that the data adequately represent seasonal growth fluctuations and particulate organics, our objectives in this phase of the study.

Relationships Between Water Flow Rate and the Growth of Pacific Oysters at Various Temperatures

Oyster growth experiments III and IV were designed for factorial analysis and included four water flow variables compounded with three temperature variables. The two experiments were conducted at different seasons to provide insight into the interaction of temperature and water flow rate, and permit consideration of a generalized relationship between water flow rate and oyster growth at different temperatures. The experiments were conducted in Newport and followed procedures previously outlined ("General Methods").

The influence of water flow rate on oyster growth at three temperatures is shown in Table 5 and graphically in Fig. 5 and 6. The absolute values obtained for growth in the two experiments were very different (Table 5), but the general form of the relationships are similar (Figure 5 and 6). Increases in water flow rate up to some maximum resulted in increases in flow rate failed to yield increased growth was lower in cold water than in warm water. The growth curves show that weight losses were greater at higher temperatures when flow was inadequate, and that there is an intermediate flow rate at which the growth curves tend to intersect each other, and growth appears to be independent of temperature.

Water flow rate requirements for oysters or other bivalves are often equated with food requirements and presented in the literature, perhaps unintentionally, as if they were universally applicable constants. Oysters obviously do not eat water, but rather require it as a food-containing medium, as a source of oxygen and minerals, and as a means of diluting and dispersing waste materials. Therefore, the water flow requirements of oysters vary inversely with the water's food content. This relationship between oyster growth and flow rate (8 ml/oyster/min in this case) is related to season (Figs. 5 and 6). Further, we have already demonstrated that the seasonality of growth in oysters is related to changes in available food.

The movement of water does affect the feeding and therefore presumably the growth of bivalve mollusks (Kirby-Smith 1972; Walne 1972). However, the effects of water movement on feeding and growth are overshadowed by the dominant effects of food density and temperature. We cannot emphasize, therefore, the absolute values obtained in our or others' studies for water flow rate requirements. We have shown that these values are seasonally variable. Most importantly, these studies do demonstrate the generalized relationships among food availability, temperature, and the growth of oysters and the profound

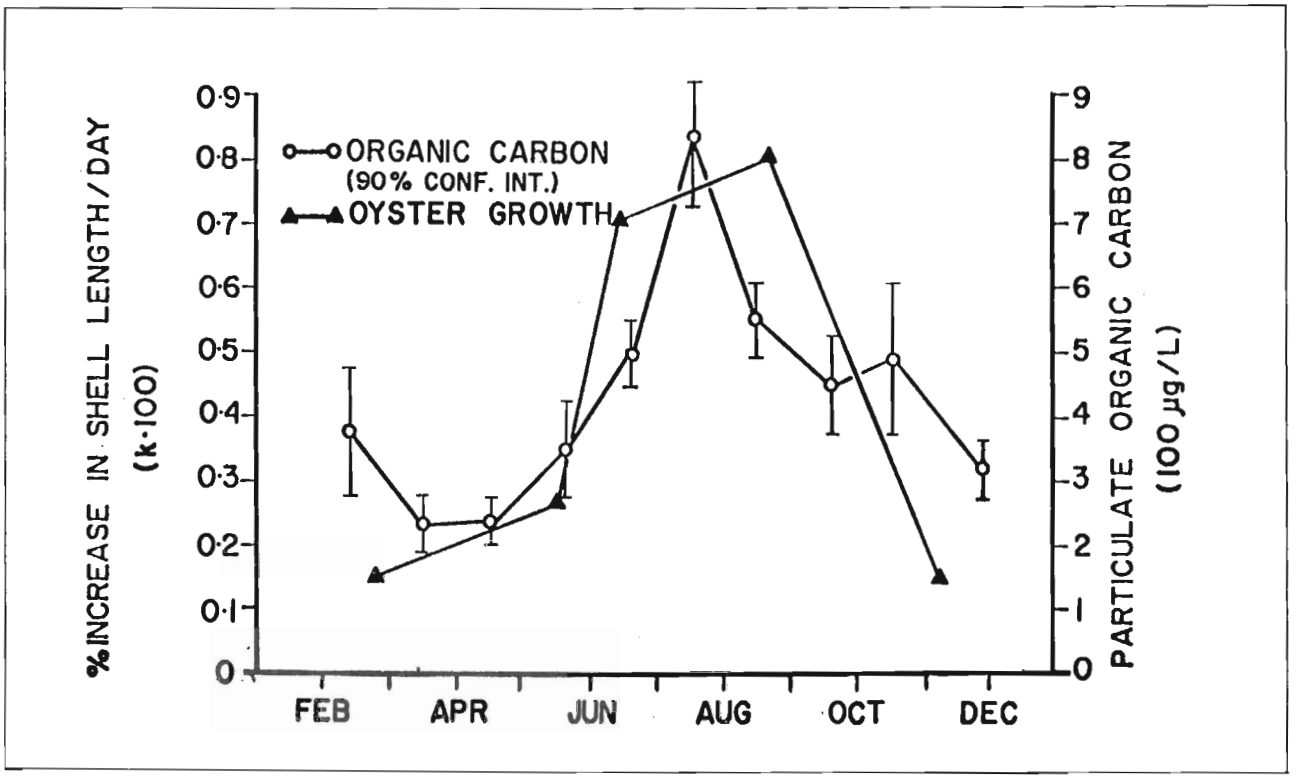

Fig. 3. Observed oyster shell growth rates from five experiments plotted at the midpoint of each experiment. Also shown are the mean particulate organic carbon values observed in the incoming sea water for each month. Carbon values are means of data collected over a two-year period.

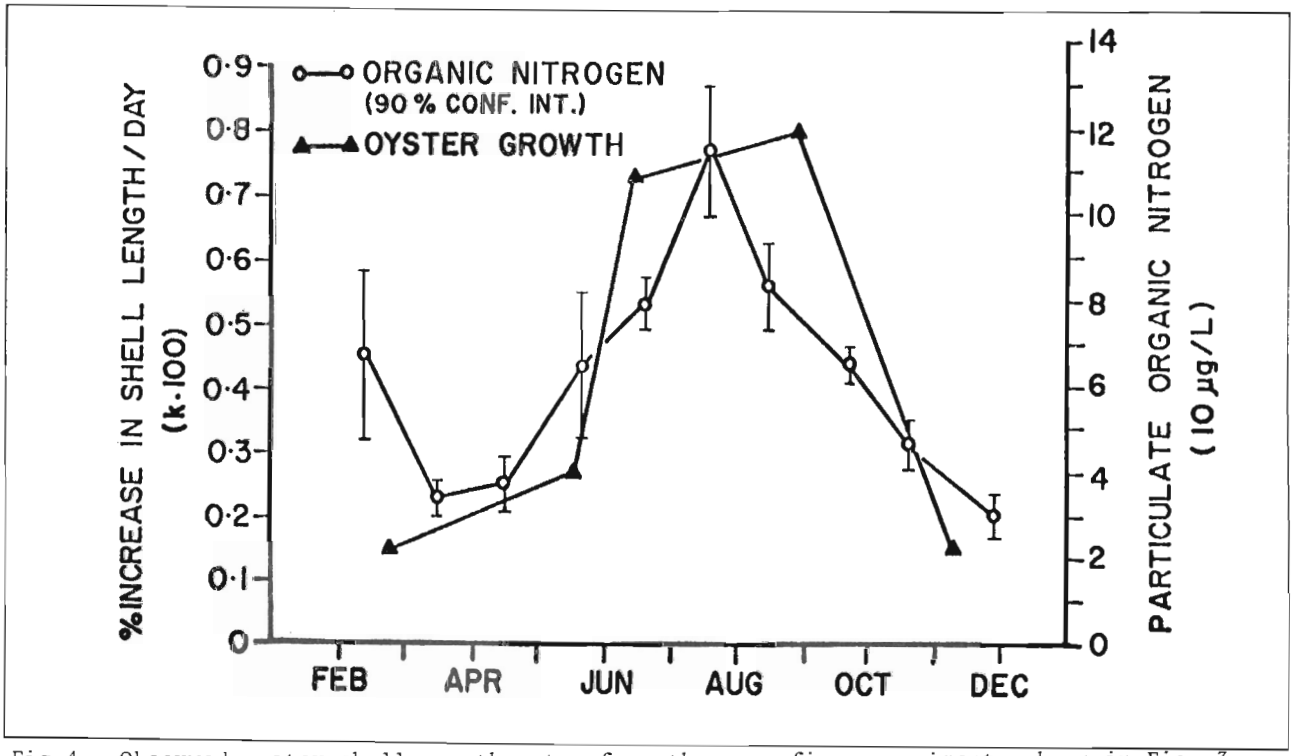

Fig 4. Observed oyster shell growth rates form the same five experiments shown in Fig. 3, but plotted in this case with monthly means of particualte organic nitrogen in the incoming seawater.

	Temp. C	Experiment III: Oct.11-Dec.15 Initial size: L=21.0 mm, Wt.=16.7 mg Water Flow (ml/oyster/min) 4	8	16	32	Experiment IV: May 15-July 17 Initial size: L=23.0 mm, wt.=23.1 mg Water Flow (ml/oyster/min) 4	16	28	40
Meat Growth (k x 100)	11	-0.72	-0.57	-0.21	+0.02	+1.06	+2.26	+2.79	+3.07
	15	-1.12	-0.41	-0.01	+0.10	+1.16	+2.55	+3.27	+3.79
	20	-0.95	-0.77	+0.11	+0.17	+0.75	+1.93	+2.97	+3.51
Shell Growth (k x 100)	11	0.03	0.06	0.13	0.13	0.52	1.04	1.03	1.09
	15	0.04	0.13	0.22	0.30	0.60	1.05	1.33	1.23
	20	0.05	0.08	0.14	0.40	0.45	0.64	1.11	1.24

(k x 100)	Temp.	Experiment IV: May 15-July 17 Initial Size: L-23.0 mm, wt. =23.1 mg Water Flow (ml/oyster/min) -0.72	-0.57	-0.21	+0.02	+1.06	+2.26	+2.79	+3.07

Table 5. The results of two factorial experiments conducted to determine the effects of temperature and water flow rate on meat and shell growth in juvenile Pacific oysters in unfiltered seawater without supplemental feeding.

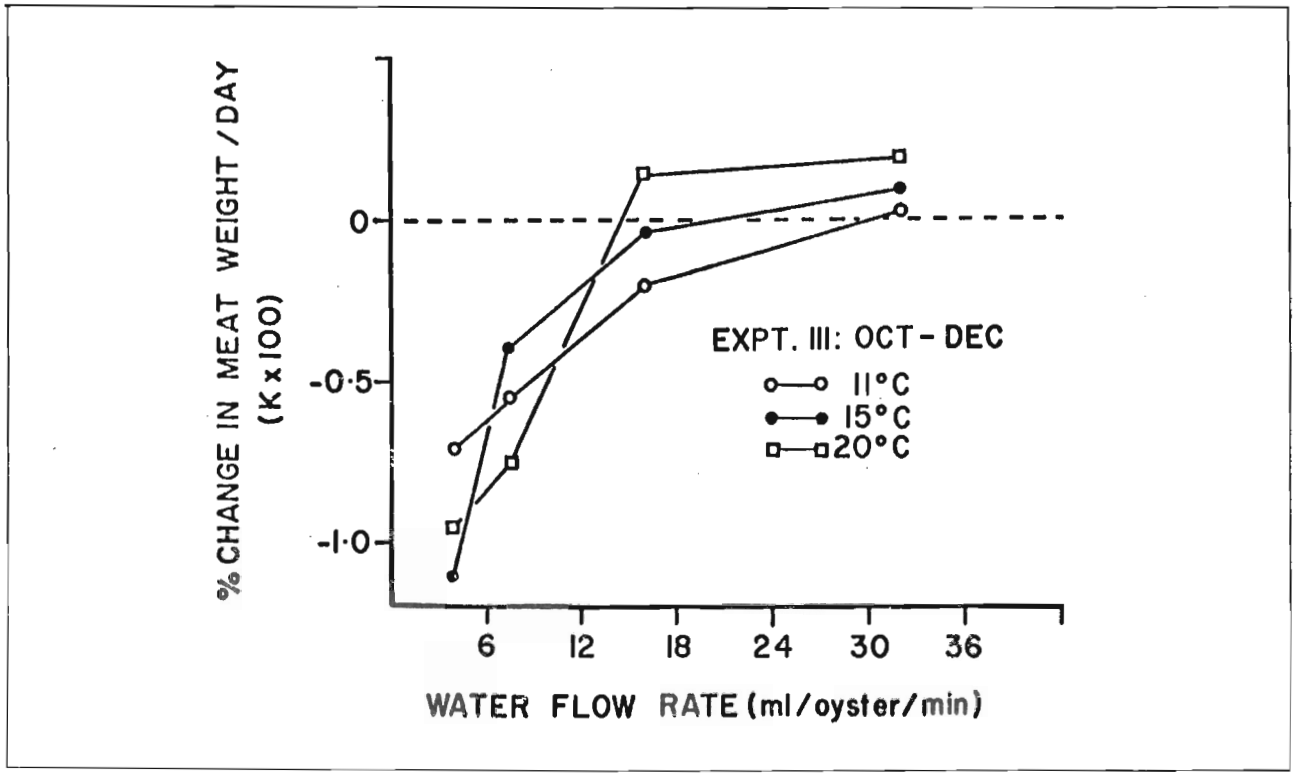

Fig. 5. The observed relationship between oyster meat growth rate and water flow rate at three temperatures. Data from Experiment III carried out October 11 - December 15.

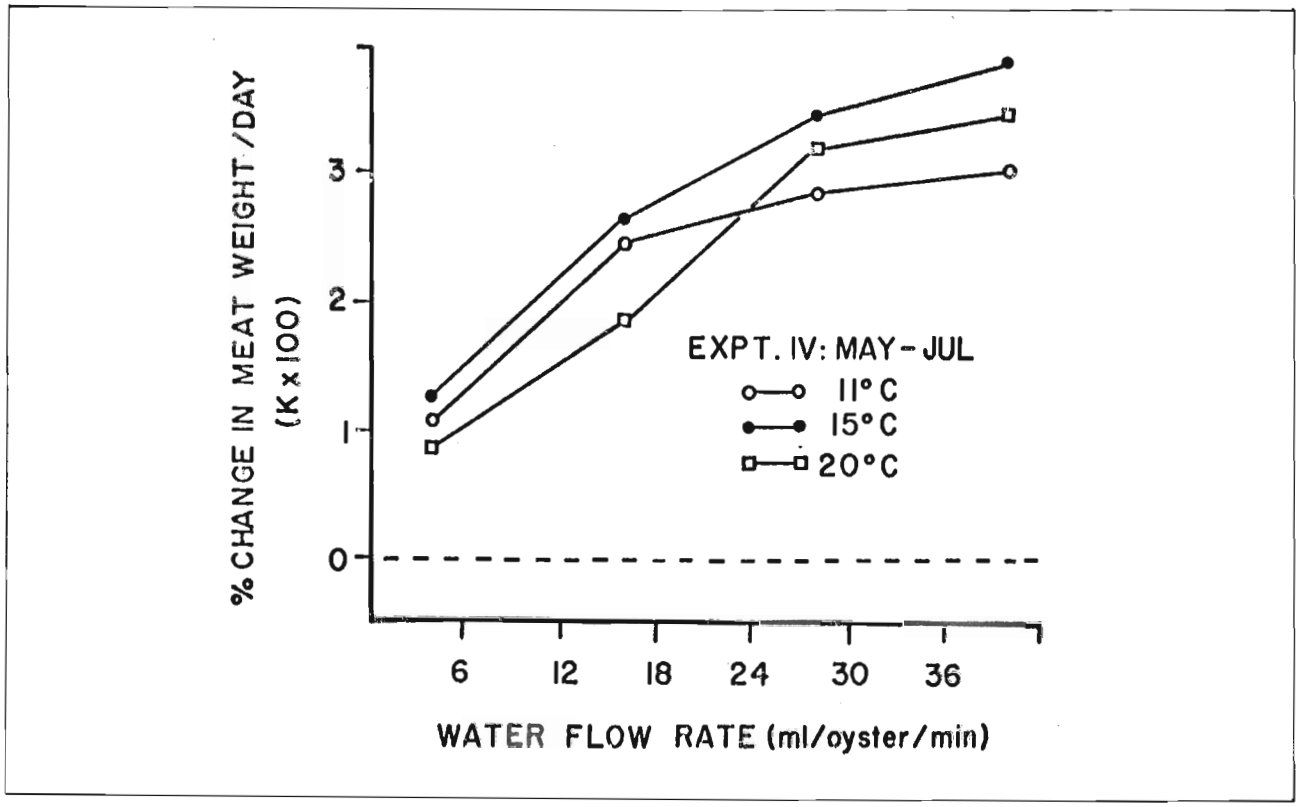

Fig. 6. The observed relationship between oyster meat growth rate and water flow rate at three temperatures. Data from Experiment IV carried out May 15 - July 17.

effect the season has on the growth of these animals.

Extreme seasonal fluctuations in the growth of oysters provided only with natural food have been shown by a number of previous studies (Askew 1972; Dame 1972; Maurer and Aprill 1973). However, these studies have generally attributed the observed fluctuations in growth rate to seasonal changes in water temperature. We have shown in our studies, conducted at a constant temperature (15°C), that temperature control alone is not necessarily sufficient to produce year-round growth in oysters, and that high temperatures during periods of low food density can create increased weight loss and high mortalities.

Definitive statements concerning the effects of temperature and water flow rate on the growth of oysters can not be made without additional information on the density of food in the incoming water. Moreover, experiments on these relationships are best conducted in an artificial system in which temperature, water flow rate, and food density can be controlled independently. Our experiments, described in the preceding section, do indicate the general form of the relationships between food availability and oyster growth at various temperatures. The hypothetical curves shown in Fig. 7 are based on Fig. 5 and 6 and on similar relationships that have been clearly demonstrated for other aquatic animals (Brett et al. 1969).

The curves in Fig. 7 have been divided into four areas (numbered 1 through 4 and enclosed by dashed lines). These four areas, corresponding to four different ranges of food availability, show four entirely different relationships between temperature and growth.

When food density is very low, there is a direct relationship between weight loss and temperature (Area 1). Alternative hypotheses can be suggested for this relationship. For example, increased weight loss at higher temperatures might be caused by increased metabolic costs in the absence of increased food availability, or food consumption relative to food density might be inhibited by high temperature. Assimilation efficienty--the percentage of food consumed that is ultimately available to the animal for respiration and growth--is reduced by increased temperature, or that the relationship is caused by a combination of the above factors.

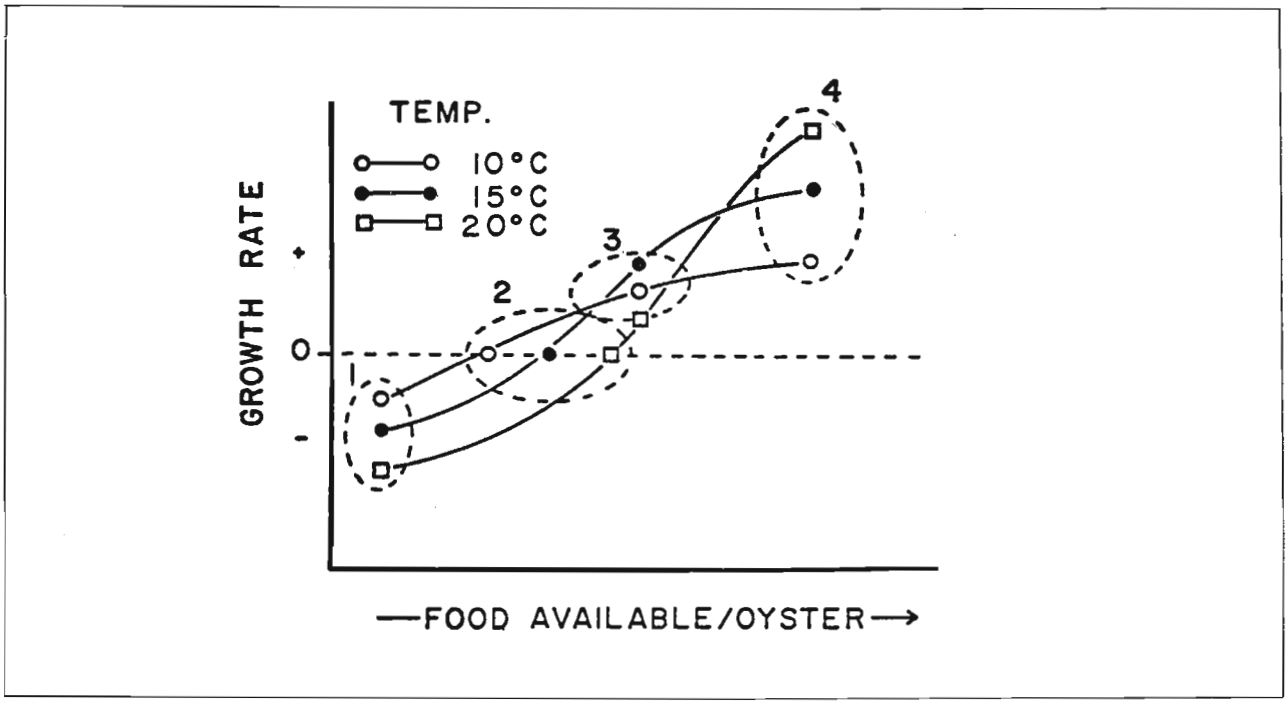

Fig. 7. Hypothetical curves relating oyster growth to food availability at three temperatures. Four areas of particular biological interest are circled with dashed lines and are discussed in the text.

Growth curves intersect the zero growth line in Area 2 (Fig. 7). In this area, food consumption balances losses to waste materials and metabolic costs, but there is no energy available for growth. The amount of food required for maintaining zero weight change apparently increases with increasing temperature. Inhibition of food consumption and increased metabolic rates at higher temperatures are probable explanations for this relationship.

Temperature-dependent growth curves intersect each other in an area of intermediate food availability (Area 3, Fig. 7). Growth appears to be independent of temperature, and relatively small changes in food density can cause a reversal in observed relationships between temperature and growth.

Finally, when food availability is very high, a direct relationship exists between temperature and oyster growth, up to some maximum temperature (Area 4, Fig. 7). In this area, further increases in food availability do not yield increased growth.

The importance of the proposed alternative hypotheses can only be evaluated in a system that permits independent manipulation of temperature and food. We designed and built such a system, and our experimental results are discussed in a following section. These results clearly indicate that the use of heated effluents for culturing the Pacific oyster would require the use of some type of supplemental food in order to be successful on a year-round basis. Experiments described in the following section also provided data on the feasibility of using cultured algae to supplement the natural food of oysters.

EXPERIMENTS IN AN ARTIFICIAL SYSTEM USING SUPPLEMENTAL FOOD

The objectives of this phase of our study were to measure food consumption, metabolic activity, and growth in juvenile Pacific oysters in a system permitting control and regulation of food availability and temperature. A secondary objective was to assess the possibility of using cultured planktonic algae to supplement the natural food of oysters.

To meet our objectives, we designed and constructed a recirculating sea water system. This system was based on a number of existing systems (Zillioux and Lackie 1969; Spotte 1970; Epifanio and Mootz 1975). Details of the recirculating system used in these studies are available elsewhere (Malouf 1977; Malouf and Breese 1977). Therefore, we present only a very brief description of the system's essentials.

The water treatment system was physically separate from the animal holding trays and temperature controls, and included a series of sequential particle filters in sequence from 116µ-5µ-3µ and finally, 0.8µ. The particle filters, which removed all of the excess food, were followed by cartridge activated carbon filters. The carbon filters were in turn followed by an ultraviolet sterilizer that reduced bacteria to acceptable levels. A conditioned biological filter of dolomite gravel kept ammonia levels at or below 0.5 ppm during our experiments.

The system was designed to maintain four different temperatures. Water was eventually recombined into a single water source and treated in a single treatment system, and then split again into four temperature control subsystems. The system provided four temperatures and permitted four levels of food availability (16 treatments), all with a common water supply. Glass heat exchangers chilled one-half of the system's 10 l/min total water flow to 15°C, and then one-half of this (2.5 l/min) was further chilled to 11°C. Another one-fourth of the system's flow was heated to 19°C, and the final one-fourth was heated to 23°C with glass immersion heaters.

Cultchless oysters, obtained from commercial hatcheries, were placed on nylon screens immersed in 41 plexiglass trays. There were 16 such trays, each receiving a flow of 200 ml/min at the appropriate temperature and containing 300 oysters. Cultured algae was metered with chemical pumps into three of the four trays at each temperature. A fourth tray at each temperature was unfed. In addition to the unfed tray, feeding rates of approximately 85, 170, and 340 µg of particulate organic carbon per liter were used at each temperature. This was accomp- with two species of algae (used on alternate days), *Monochrysis lutheri* and *Pseudoisochrysis paradoxa*. The feeding rates given above correspond approximately to 10,000, 20,000, and 40,000 cells per milliliter of *M. lutheri*, and 14,000, 28,000, and 56,000 cells per milliliter of *P. paradoxa*. Algae used in the study were clultured in 60 l plexiglass tanks using techniques described by Breese and Malouf (1975). Algae were fed only from cultures in an active growth phase (the logarithmic portion of the growth curve).

The Energy Budget

Our objective in this part of the study was to determine an "energy budget" for juvenile Pacific oysters, and to ascertain how that energy budget is affected by food density and temperature. The idea of an energy budget derived from an effort to simplify a complex subject. Irlev (1945) and Fry (1947) pioneered the approach for aquatic organisms, and Warren and Davis (1967) refined it. The concept is essentially an attempt to list and to measure all of the possible fates of the energy content of food eaten by an animal. The approach views growth as just one of a number of possible fates of the energy of consumed food. Determination of the relative magnitudes of other energy costs and consideration of the effects of environmental factors on the partitioning of energy provides the basis for understanding, not just oberving, growth.

The energy budget equation proposed by Warren and Davis is, in its simplest form, as follows:

$$Q_c - Q_w = Q_g + Q_r,$$

in which

Q_c = the energy content of food consumed by an animal

Q_w = the energy content of all waste products (feces, urine, etc.)

Q_g = the energy content of growth (soft tissues, shell, gonad, etc.)

Q_r = the energy lost through respiration (metabolic costs)

The Warren and Davis equation was used without modification by Thompson and Bayne (1972) in their study of growth in the mussel, *Mytilus edulis*. However, some minor modifications may make it more useful and meaningful for studies of bivalve molluscs. For example, bivalves are able to clear great quantities of particulate matter from suspension, but their food handling mechanisms are more limited in capacity. Consequently, some fraction of material cleared by an oyster may be complexed in an amorphous, mucus-bound mass called pseudofeces (Bernard 1974). Therefore food consumption may be more meaningfully thought of (Q_c) as the arithemtic difference between food cleared (Q_{cl}) and pseudofeces (Q_p).

Most studies of growth in bivalve molluscs have considered the energy content of the soft tissue and have ignored the energy content of the shell's organic fraction. A few studies have attempted to measure energy contained in the organics of mollusk shells (Hughes 1970; Dame 1972:); others have assumed shell organics to be negligible (Tenore et al. 1973). The shell organics may constitute only about 1-2 percent of the weight of the shell, but this material may be significant relative to the weight of the body of the animal. Shell organics should be considered in the energy budget as part of growth, since little evidence supports the assumption that they have an insignificant energy content.

Finally, since respiration varies with food consumption rate (Thompson and Bayne 1972), respiration should be measured under conditions that are as similar as possible to those under which growth is determined.

Our study involved the direct measurement of food cleared (Q_{cl}), respiration (Q_r), and growth (Q_g). Since no pseudofeces were formed in any of the experiments, $Q_c = Q_{cl}$. Waste production was estimated as the difference between assimilation ($Q_g + Q_r$) and food consumption (Q_c). Assimilation efficiency, a measure of the fraction of food consumed that is available to the animal for respiration or growth, was defined in this study as

$$\frac{Q_g + Q_r}{Q_c} \times 100 .$$

Methods for Determining the Components of the Energy Budget

The rate of the oysters' food consumption was determined by making repeated counts of the density of algal cells entering and leaving the trays containing the animals. A Coulter Counter model ZBI, which was used included a P-64 Channelizer and an X-Y plotter, for rapid and accurate determinations of algal cell density and the size distribution of the algal cells. When no oysters were present in the trays, the inflow and outflow densities differed by an average of only 2 percent. Moreover, the size distribution of the cells was not altered as the cells passed through the trays, even though the temperatures in those trays (11°, 15°, 19°, and 23°C) were different from the temperature in the system's algal reservoir (17°C). When animals were present, the

outflow density was generally from 40 to 80 percent of the inflow density.

In one trial, counts of the inflow and outflow densities were made (with oysters present) every two hours for 20 hours. These counts did not show any pattern of feeding activity or inactivity among the population of oysters as a whole. That is, the oysters' rate of removal of algal cells was relatively constant during the 20-hour period, despite the fact that the oysters were in the dark for 12 hours and in room lighting for 8 hours of that time.

During the course of the experiments, we noted that the condition of the algal cells changed considerably during the period of time that they were stored in the reservoir. After 20 hours in the reservoir, for example, *Pseudoisochrysis paradoxa* cells lost 19 percent of their organic carbon, 18 percent of their nitrogen, and 40 percent of their mean cell volume. These changes in cell volume were taken into account in our calculations of food availability and consumption. However, algal cells should not be thought of as constant units of food. Food consumption of food density in terms of algal cells has no significant meaning unless the nature and size of those cells is well characterized.

Dichromate wet oxidation procedures (Maciolek 1969, Strickland and Parsons 1972) were used to determine the caloric value of the algal cells. Our data yielded a conversion factor of 9.22 calories per mg of carbon for *P. paradoxa* and 9.65 calories per mg carbon for *Monochrysis lutheri*.

Oxygen consumption was determined in small jars arranged to siphon off a flow of water from the inflow to a particular tray. Oysters were placed in three of five jars at each treatment condition. Water with food (except in the case of the unfed groups) was allowed to flow through the jars for 18 hours. The jars were then sealed, and were allowed to stand at the appropriate temperatures for two hours. The dissolved oxygen concentration in the jars was then determined using Winkler titrations. The difference in dissolved oxygen between the jars containing oysters and the control jars that did not contain oysters indicated the oxygen consumption of the animals. Oxygen consumption was converted to caloric terms using an oxycalorific coefficient of 3.42 calories per mg of oxygen (Warren 1971).

The growth of the animals was determined by comparing the ash-free dry weight of the animals at the end of an experimental period with the ash-free dry weight of a randomly selected subsample taken at the beginning of the experiment. Preliminary trials showed that hand shucking these very small oysters (3-10 mm) was extremely difficult without introducing significant errors from shell fragments or from incomplete recovery of the meats. Therefore, an alternative shucking technique using concentrated HCl was devised and tested.

The acid shucking technique permitted rapid handling of large numbers of very small animals, and, more importantly, it permitted recovery of the acid-insoluble organic shell matrix along with the meat. Preliminary trials showed that the acid had no effect on the dry weight or ash-free dry weight of hand-shucked oyster meats (although the tissues were obviously denatured by the acid). These trials also showed that the caloric content of the meats was unaffected by the treatment, and that the residual $CaCl_2$ produced by the reaction of the acid with the $CaCO_3$ of the oyster shell could be removed by a five-minute distilled water wash.

Our data showed that the recovered and weighed organic shell matrix amounted to approximately 20 percent of the total organic content of 6-10 mm oysters. Omission of this material from energy budget estimates would introduce a significant error.

Dichromate wet oxidations were performed on the acid-shucked meats and shell matrices to provide a conversion of ash-free dry weights to calories. These determinations yielded results that were somewhat lower than those reported by other workers (Russell-Hunter et al. 1968; Dame 1972), but the very small animals used in our study are known to be low in lipid content and therefore in caloric content. Moreover, these animals have a high percentage of protein, a substance that is more resistant to dichromate oxidation than either carbohydrate or lipid. These determinations yielded a mean of 4081 calories per gram of ash-free dry weight of oyster tissue, including the proteinaceous shell matrix.

For the energy budget experiments, growth rate was calculated from the equation:

$$\text{Growth Rate} = \frac{W_2 - W_1}{(0.5)\ (W_1 + W_2)\ (t_2 - t_1)},$$

in which

W_2 = the mean caloric content per animal at the end of the experiment

W_1 = the mean caloric content per animal at the beginning of the experiment

$(t_2 - t_1)$ = the duration of the experiment in hours

The equation expresses an increase in caloric content, relative to the mean caloric content of the animals in each treatment, during the course of the experiment. The other components of the energy budget equation were also expressed relative to the mean caloric content of the animals. Growth rates so calculated are expressed in terms of calories per calorie hour. These were multiplied times 1,000 to yield calories per kilocalorie per hour.

Results

In conducting oyster growth experiments in a recirculating system supplied with cultured algae as a source of food, our objectives were primarily to determine how temperature and food density influenced the components of the animals' energy budget. Concurrently, we investigated the feasibility of using cultured algae to supplement or substitute for the natural food of oysters.

Food Consumption (O_c): Results of our food consumption determinations demonstrate a clear difference in the rate at which the animals consumed the two different species of algae (Table 6). In every treatment, but particularly at higher temperatures, the oysters consumed much more of the *Pseudoisochrysis paradoxa* than they did of *Monochrysis lutheri*. At 23°C, for example, the oysters consumed an average of 6.4 percent of the *P. paradoxa* but only 2.7 percent of the *M. lutheri* available to them. These two species of algae, superficially very similar, are both flagellates with a diameter of 3-5 µm, and both have been shown to support good growth in oyster larvae.

Evidence in the literature indicates that bivalves do show different responses to different species of algae. For example, Davids (1964) found that the mussel, *Mytilus edulis*, consumed different species of cultured algae at very different rates. In fact, his results led him to conclude that the mussels "did not like algae of the genus *Chlorella* as food".

These data underscore the problems involved in general statements concerning rates of food consumption in oysters fed cultured algae. As we have already noted, food consumption data in terms of algal cells are difficult to interpret, because algal cells even of a single species can vary widely in weight, volume, and food content. Moreover, the species of algae involved clearly has a major bearing on the oysters' rate of consumption of the algal cells (Table 6).

We found that food consumption ranged from a low of about 10 percent to as high as 50 percent of body weight per day. These values are higher than literature reports based on studies involving adults and juveniles of the mussel, *Mytilus edulis* (Bayne 1976). However, Bayne's data clearly show an inverse relationship between body weight and food consumption as a percentage of body weight. Moreover, animals generally consume a lower percentage of their body weight per day as that weight becomes greater (Warren 1971). Data from additional experiments (Malouf 1977) showed that larger oysters did consume a lower percentage of their body weight per day. For example, at 15°C oysters have an ash-free dry weight of about 3.0 mg fed *P. paradoxa* consumed an average of 10 percent of their body weight per day. This compares to an average of 41.6 percent of body weight consumed per day by the 0.1 mg oysters. Bayne (1976) reported that mussels having ash-free dry weights of 200 mg consumed 6-13 percent of their body weight per day, and that 2.0 g animals consumed only 1-3 percent of their body weight per day.

Additional data is required before precise predicitons of food consumption can be made for oysters of varying sizes. However the relationship between food weight specific consumption and body weight is clearly inverse. Food consumption probably ranges from a high of about 50 percent of body weight per day for very small spat to a low of about 2 percent of body weight per day for adult oysters.

Generally, the rate of food consumption in absolute terms was directly related to food density (Table 6), but this was not true for animals fed *M. lutheri* at high temperatures. Food consumption as a percentage of food available decreased as density increased (Table 6). This results

Temp C	Tray No.	*P. paradoxa* Available (cells/ml)	*P. paradoxa* consumed: Cells/ Oyster/ Minute	*P. paradoxa* consumed: % of food Available	*P. paradoxa* consumed: % of Body Weight Per day	*M. lutheri* Available (cells/ml)	*M. lutheri* consumed: Cells/ Oyster/ Minute	*M. lutheri* consumed: % of food Available	*M. lutheri* consumed: % of Body Weight Per Day
11°	1	42,798	3809	8.6	46.5	40,664	1609	4.9	32.7
	2	22,832	2443	10.6	31.2	20,138	1347	8.1	28.7
	3	11,989	1160	11.6	19.4	8,152	481	5.4	13.3
	4	unfed	----	----	----	unfed	----	---	----
15°	5	55,131	5501	12.0	52.2	40,275	1716	8.6	45.2
	6	27,054	3412	15.1	39.1	20,344	1426	8.5	27.2
	7	13,129	2642	24.3	33.5	10,172	1135	13.5	24.0
	8	unfed	----	----	----	unfed	----	----	----
19°	9	56,077	4034	8.6	44.7	39,863	557	1.7	10.3
	10	28,494	3628	15.4	48.1	20,756	978	5.9	21.6
	11	13,639	1078	9.6	18.4	9,760	1038	12.9	29.3
	12	unfed	----	----	----	unfed	----	----	----
23°	13	53,222	1971	4.4	33.9	41,100	464	1.4	13.3
	14	29,669	1983	8.0	39.7	19,725	420	2.6	14.0
	15	16,639	950	6.9	19.5	10,790	350	4.1	12.0
	16	unfed	----	---	----	unfed	---	---	----

Table 6. Consumption of *Pseudoisochrysis paradoxa* and *Monochrysis lutheri* by juvenile Pacific oysters held in a recirculating system at four temperatures and three algal densities in flowing seawater. The oysters had an initial mean ash-free dry weight of about 100 µg and were about 3.2 mm in length.

in a higher percentage of food wastage in a once-through system, but may or may not be balanced by increased growth at higher food densities.

Food consumption data are presented in terms of calories per kilocalorie per hour (Table 7). These data are weighted means that account for the number of hours that the animals were fed the two algal species during the experiment and for the changes in the caloric content per cell that occurred in the system's algal storage reservoir.

Oxygen Consumption (Q_r): The rates of oxygen consumption measured in the experimental animals varied considerably, particularly among the unfed animals. Oxygen uptake rates determined early in the eight-week experiment were compared to rates determined after six weeks, and show that the oysters did partially acclimate to increased temperatures. Animals removed from water of ambient temperature (about 12°C) and placed in water at 23°C showed a large increase in oxygen uptake. This was partially an acute response to the temperature increase, and oxygen uptake declined among those animals as the experiment progressed until oxygen uptake determinations for animals held at the four temperatures showed less significant differences (Table 7). There was little evidence of complete acclimation, however, and oxygen consumption among animals held at temperatures up to 19°C continued to be higher than consumption in animals held at 11°C. Animals held at 23°C showed reduced metabolic activity later in the experiment. This reduction in activity--shown in both food and oxygen consumption--was probably a response to stress.

Assimiliation Efficiency and Waste Production (Q_w): The energy budget plots show that waste production accounts for most of the energy from the food consumed by the oysters (Fig. 8). Conversely, assimilation efficiencies are quite low, amounting to 22 to 42 percent of the food consumed (Table 7). Assimilation efficiency values as high as 90 percent for bivalve mollusks fed cultured algae occur in the literature (Thompson and Bayne 1972; Langefoss and Maurer 1975). However, studies involved large animals, and assimilation efficiency estimates were obtained by comparing the ash or caloric content of the feces with that of the food. This technique, called the "Conover ratio" makes some assumptions that may not be valid. In any case, microscopic examination showed that the feces produced by oysters in our experiments contained a large number on intact algal cells, many of which retained their motility.

An inverse relationship exists between food density and assimilation efficiency, although this relationship is not well defined for *C. gigas* at the higher temperatures. This inverse relationship between food density and the assimilation efficiency of food consumed has been shown for bivalves in other studies (Winter 1970; Thompson and Bayne 1972).

Growth (Q_g): The growth rates of the animals were directly related to food density at each temperature (Table 6). Maximum growth was observed at 15°C, and maximum weight loss (negative growth) occurred at 23°C. No positive growth was observed at 23°C at any of the food densities used.

Partial energy budgets for oysters held at the four experimental temperatures and four food densities are shown in Fig. 9. These plots omitted food consumption and waste to permit use of a larger scale than Fig. 8. The curves show a general sigmoidal relationship between food availability was very low or very high, growth rate appeared relatively insensitive to further changes in food availability.

At low to moderate food densities, respiration rate is directly related to food density (Table 7). Assimilation efficiency, on the other hand, is inversely related to food density. The negative effect on growth of declining food density is partially offset by decreased metabolic costs (respiration) and by increased assimilation efficiency. The net effect is stabilization of weight change over a broad range of food densities.

At very high food densities, respiration rate may stabilize (Bayne 1976). Moreover, as food density increases both food consumption as a percentage of food available and assimilation as a percentage of food consumed decline. Consequently, at very high food densities, growth may be unaffected by relatively large changes in density.

Maintenance Requirements For our purposes, maintenance requirements are defined as the energy that must be available, consumed, or assimilated so that respiration is just balanced by assimilation, and

Temp C	Tray No.	Food Available (cal/kcal/hr)	(Q_c) Food Consumed (cal/kcal/hr)	(Q_r) Oxygen Consumed (cal/kcal/hr)	(Q_g) Growth Rate (cal/kcal/hr)	(Q_w) Waste (cal/kcal/hr)	Assimilation Efficiency (percent)
11°C	1	240.7	17.35	3.47	+0.39	13.49	22.3
	2	132.2	12.60	2.85	+0.35	9.40	25.4
	3	82.4	7.29	2.14	-0.04	5.19	28.8
	4	unfed	-----	0.61	-0.04	-----	----
15°C	5	193.7	20.67	4.09	+0.65	15.93	22.9
	6	116.7	14.50	3.60	+0.47	10.43	28.1
	7	65.0	12.50	3.69	+0.36	8.45	32.4
	8	unfed	-----	1.84	-0.20	-----	----
19°C	9	223.5	13.43	4.54	+0.51	8.38	37.6
	10	144.7	15.31	3.84	+0.36	11.11	27.4
	11	85.6	9.27	3.51	-0.04	5.80	37.4
	12	unfed	-----	1.84	-0.14	-----	----
23°C	13	394.4	11.01	3.48	-0.05	7.58	31.2
	14	213.9	12.66	3.42	-0.31	9.55	24.6
	15	117.7	6.96	3.34	-0.36	4.00	42.8
	16	unfed	-----	0.55	-0.56	----	----

Table 7. An energy budget summary for juvenile oysters, *Crassostrea gigas* (Thunberg), held in a recirculating system at four temperatures and four levels of food availability. The oysters had a mean initial shell height of 3.2 mm and a mean initial ash-free dry weight (including shell organics) of 0.102 mg.

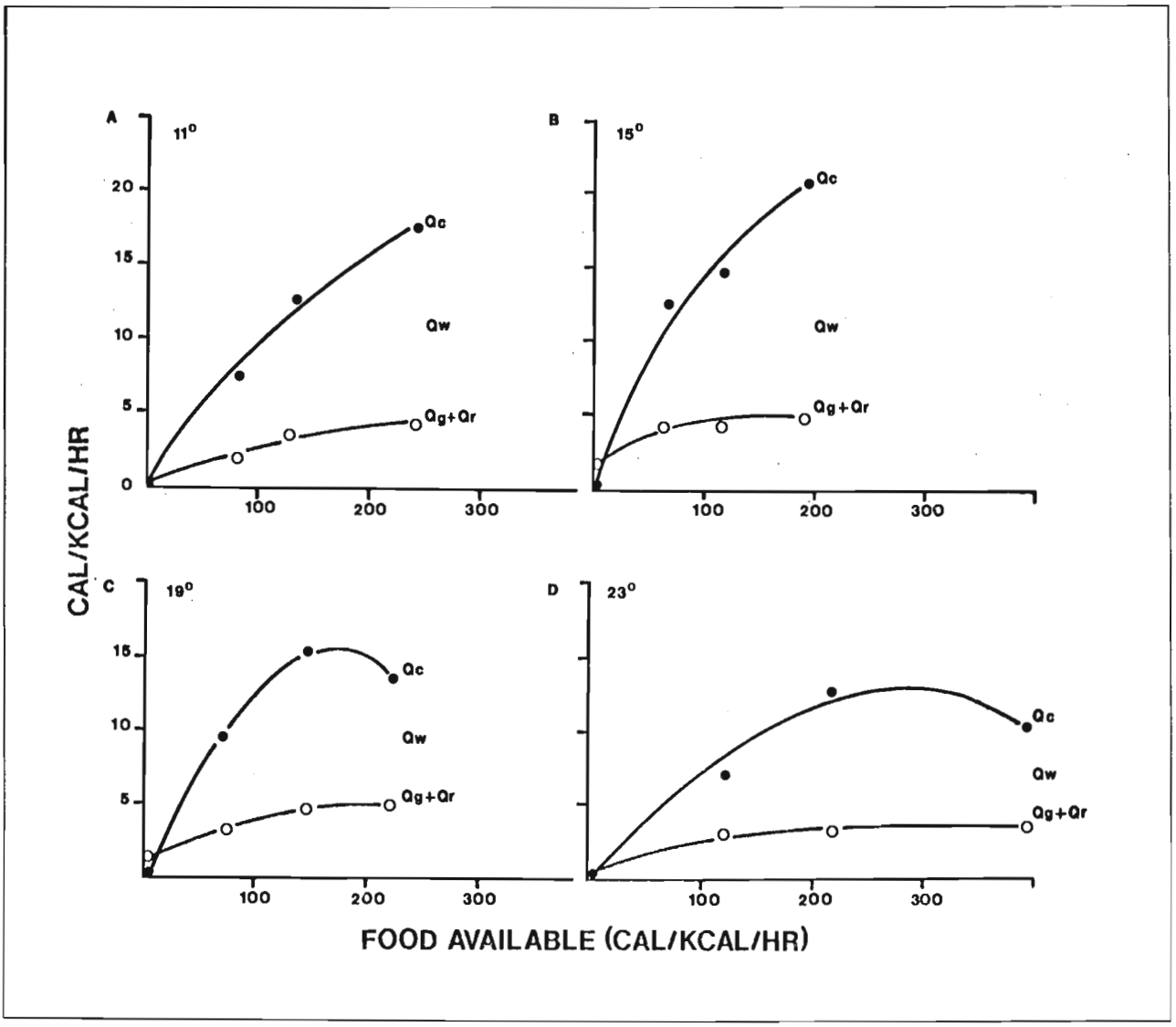

Figure 8. Graphical representation of the energy budgets of juvenile oysters held at four temperatures and four ration levels in "Closed system" Experiment 11. Q_c = food consumption rate; Q_w = rate of waste production; $Q_g + Q_r$ = assimilation rate; and Q_r = respiration rate.

growth is zero. Maintenance requirements in terms of food availability may be graphically estimated at the intersection of the growth curves with the zero growth rate line (horizontal dashed line in Fig. 9). Maintenance requirements in terms of assimilation may be graphically estimated as the point at which the respiration and assimilation curves intersect each other (Fig. 9).

Graphical estimation of maintenance requirements is accurate only if the shapes of the curves involved are well defined. For example, the growth curve for oyster held at 15°C has only a single point below the zero growth line (Fig. 9). The curve thus lacks the characterisitc sigmoidal shape, and graphical interpolation underestimates the maintenance requirements at 15°C.

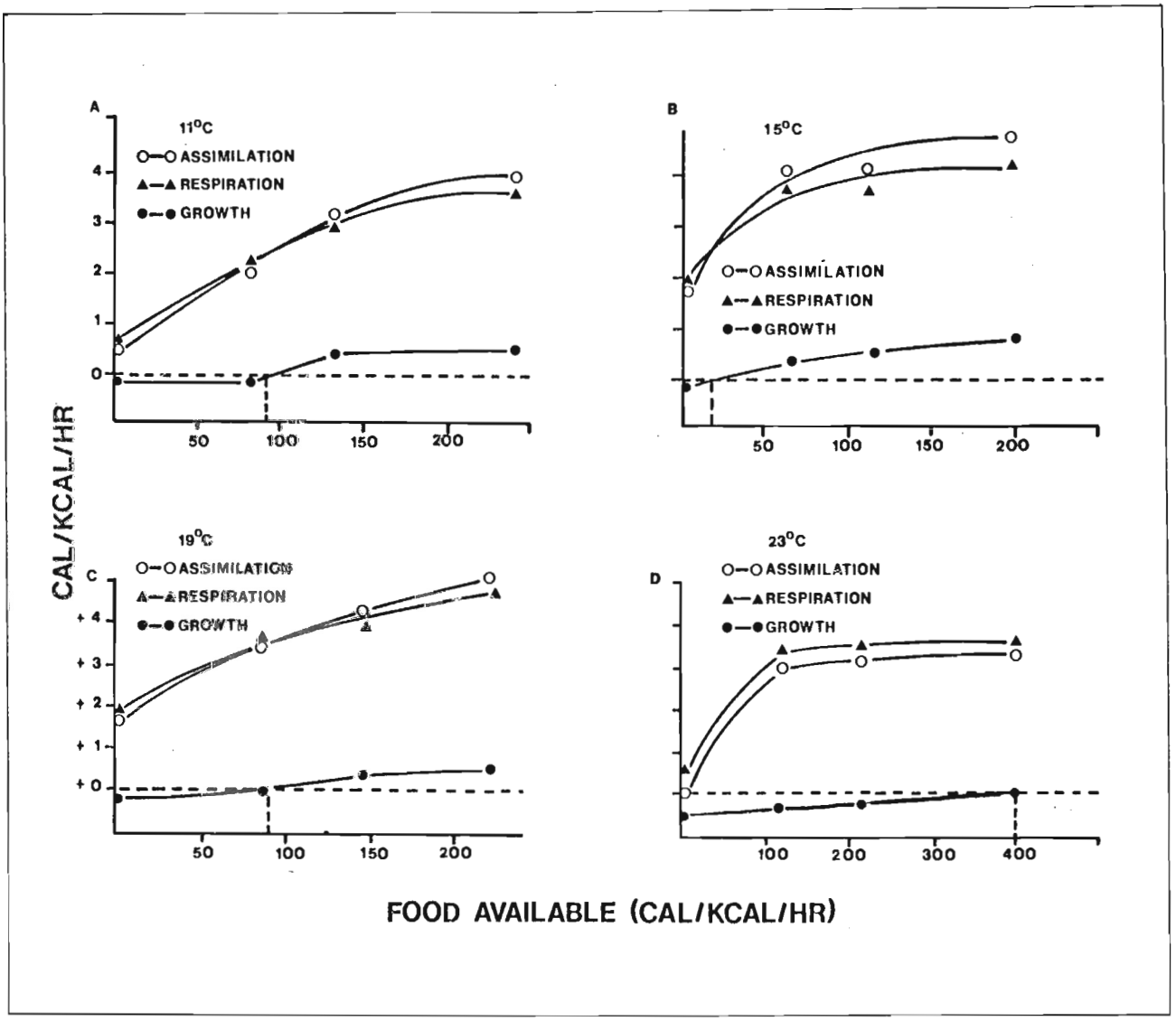

Figure 9. A partial energy budget (Q_c and Q_w omitted) for juvenile oysters held at four different temperatures and fed four different ration levels. Graphical estimate of food availability necessary to maintain zero weight change as shown.

Estimates of maintenance requirements can also be calculated from the energy budget equation. By letting growth (Q_g) equal zero, and estimating respiration (Q_r), assimilation, and food consumption--in absolute terms and as a percentage of availability--from the data, maintenance requirements in terms of food availability can be estimated. Maintenance in terms of food consumption can be similarly estimated. Maintenance in terms of assimilation can be assumed to equal the respiration rate of animals under conditions that yield zero weight change.

Maintenance requirements for oysters held at 11°, 19°, and 23°C are graphically estimated in Table 8. Estimates given for 15°C are calculated values, and only the value for availability differed significantly from the graphical estimates, which was 25 cal/kcal/hr.

Temp. C	Maintenance Requirement (cal/kcal/hr) Availability	Consumption	Assimilation	Estimated Assimilation efficiency at zero weight change (percent)
11	92	8.4	2.3	27
15[1]	40	7.9	2.6	38
19	93	10.6	3.4	32
23	390	12.0	3.5	32

[1] Values for 15°C were calculated as described in the text.

Table 8. Graphically estimated requirements to maintain zero weight change at four temperatures. Requirements presented in terms of food availability, food consumption, and assimilation.

These estimates show that maintenance requirements in terms of food availability reach a minimum at 15°C (Table 8). Maintenance requirements in terms of assimilation, on the other hand, are directly related to temperature (Table 8 and Fig. 10). Measured assimilation reached a maximum at about 15°C and declined with further increases in temperature (Fig. 10, data from Table 7). The arithmetic difference between measured assimilation rate and estimated assimilation required to maintain zero weight change was greatest at 15°C.

The highest temperature at which positive weight change could be expected under the conditions of the experiment and at the lowest food density (88 cal/kcal/hr) was about 18°C (Fig. 10). An approximate doubling of the ration to 152 cal/kcal/hr increased the maximum temperature for positive growth to about 22°C. A further increase in food density) to 252 cal/kcal/hr) did not result in an increase in the maximum temperature at which positive growth could result. The oysters showed positive growth over a broader range of temperatures when food was plentiful than when it was scarce (Fig. 10), but an upper temperature limit--and presumably a lower temperature limit--exists that is independent of the quantity of food available to the oysters.

Maintenance requirements in terms of assimilation incrase with increasing temperature, but at temperatures over 15°C there was very little increase in food consumption as a percentage of food available. Consequently, as temperature increased, maintenance requirements in terms of food availability increased disproportionately to increases in metabolic costs. Approximately a threefold increase in food availability was required to maintain zero weight change with each increase of four degrees Celsius.

Discussion Before considering the implications of these results for the culture of Pacific oysters in heated effluents, we will touch on a number of specific points that warrant discussion.

First, the arithmetic difference between estimated maintenance in terms of assimilation and measured assimilation (Fig. 10) is not "scope for growth." Scope for growth, as defined by Warren and Davis (1967), and applied to studies of mollusks by Thompson and Bayne (1972), is the energetic difference between assimilation and respiration. Maintenance requirements estimated from growth curves provide a measure of the amount of energy required under given conditions to maintain zero weight change indefinitely (Fig. 9). In that sense they are useful for considering the effects of temperature on animals.

However, maintenance requirements estimated in this manner are equivalent to respiration only under conditions yeilding zero growth. Clearly, respiration is not independent of food consumption, and no single maintenance requirement estimate can be valid at all rates of food consumption for estimating scope for growth.

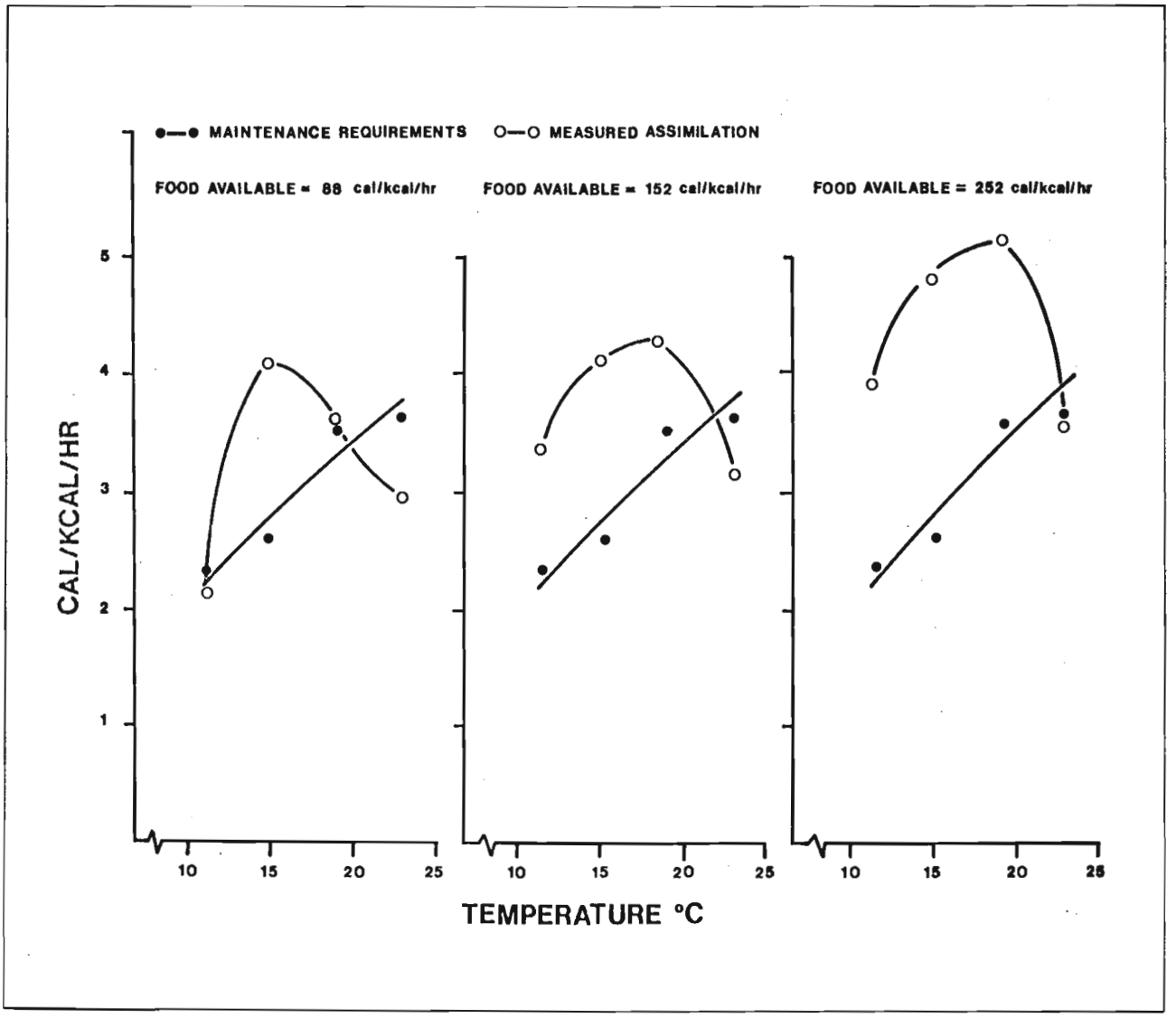

Figure 10. The relationship between temperature and the estimated assimilation rate required for zero weight change shown with the measured assimilation rate at each temperature. This relationship is shown for each of three different levels of food availability. Data from closed system Experiement 11.

Secondly, the results of our study indicate that the optimum temperature for the growth of juvenile Pacific oysters is about 15°C. The data also show that no positive growth can be expected at temperatures in excess of 22°C regardless of the food density. However, *C. gigas* does grow under some conditions at temperatures exceeding 22°C (see Fig. 1). This apparent discrepancy suggests that growth conditions in our experiments were not optimum for some factor other than food density or temperature. We feel that the system did support good growth (increases of up to 150 percent in eight weeks). However, these results or any other growth results should be applied with care to other environmental systems in the absence of additional information on the effects of a variety of factors on oyster growth.

Thirdly, the expression of food availability in terms of calories provides a useful common denominator for discussing the results of energy budget experiments. However, the use of caloric equivalents may obscure important qualitative differences among food materials. We observed clear

differences in the rates at which the oysters consumed the two species of algae used, despite the fact that these algal species provided them with essentially the same caloric value. These results may indicate that the energy budget, and therefore the growth of oysters, is influenced by qualitative differences in food, and that food consumption rate is at least one of the components of the energy budget that is affected.

summary and recommendations

We have reviewed the results of a large number of experiments and, to a lesser extent, have discussed some pertinent information published in other sources. In the following sections, our findings are summarized and used in combination with other work to draw some general conclusions concerning the biological feasibility of intensively culturing Pacific oysters in heated effluents. Finally, we recommend possible designs for such a culture system and suggest critical areas in which additional information is needed.

SUMMARY

Our studies dealt with the biological feasibility of using heated effluents to culture Pacific oysters, *Crassostrea gigas*, to market size. We did not consider possible uses of heated effluents in traditional extensive culture methods (as in culture of oysters using traditional methods in the thermal plume of a power plant), in hatchery operations. Our studies were concerned only with biological feasibility.

Even a cursory review of published accounts provides sufficient background to indicate that the intensive culture of oysters is in general biologically feasible. However, several important questions remain in doubt. Can beneficial use be made of increased temperature on a year-round basis? Is it biologically feasible to feed oysters when their natural food is scarce. What are the optimum conditions of temperature and food density for the growth of Pacific oysters? Our study attempted to answer these questions and others.

Since it appears unlikely that future power plants will be constructed on any of Oregon's limited estuarine waters, we needed to determine if Pacific oysters could survive and grow in full-strength seawater from an open coastal location. Experiments comparing the growth and survival of oyster spat in water from the Yaquina estuary in Newport and in water from the open coast at Port Orford showed that full-strength seawater is not inherently harmful for culturing Pacific

oysters. However, an open coastal site is likely to provide less food for the oysters than a highly productive estuary, and some provision may have to be made for supplemental feeding of the oysters.

A series of experiments showed little or no growth advantage to temperatures in excess of 15°C. However, since ambient coastal temperatures range from 9°C to about 12°C, some growth benefit could be obtained by warming the water.

High temperatures (above 20°C) resulted in reduced growth and high mortalities, particularly if high temperatures were accompanied by low water flow rate or low natural food availablility.

Extreme seasonal fluctuations in the growth of oysters under constant conditions of water flow rate and temperature were observed. Little or no growth was recorded between the months of October and March (five to six months).

In a series of five experiments, oysters of the same initial size were held at a flow rate of 8 ml/oyster/min at 15°C during different times of the year. Concurrently particulate organic carbon and nitrogen in the natural seawater were monitored to provide an indication of available food for the oysters. These experiments showed that: (1) the fluctuations in oyster growth rate were not caused by temperature changes; (2) increased temperatures alone would not produce good growth in oysters during the winter months; (3) particulate organic carbon and nitrogen appear to provide an adequate indication of natural food availability; and (4) the growth of oysters is minimal during the winter because food is scarce.

Experiments showed that there is no single relationship between water flow rate and oyster growth at different temperatures, because the water flow requirements are ultimately determined by the food content of the water. Food density in the water demonstrated extreme seasonal fluctuations, which are reflected in changes in water flow requirements.

During the winter, no growth was observed at high temperatures regardless of the flow rate, because the oysters' feeding is limited ultimately by their ability to filter the water, not by the rate of flow of water past them.

Experimental results permit us to suggest general hypothetical curves that relate food availability to oyster growth rate at various temperatures. These curves show how growth rate is inversely related to temperature under some conditions, and is either directly related or seemingly unrelated to temperature under other conditions.

Since the use of elevated temperatures to enhance the growth of oysters depends for success on food availability, we conducted experiments that included among their objectives determination of the biological feasibility of supplementing natural food supplies with cultured algae.

These experiments, conducted in a recirculating seawater system provided with measured amounts of cultured algae, also enabled us to develop energy budgets for juvenile oysters at different temperatures and food densities.

Results showed that juvenile oysters could be grown on a diet of cultured algae and reaffirmed the 15°C optimum temperature for the growth of juvenile Pacific oysters.

The food consumptions of the oysters depended on the species of algae used. The oysters consumed much more *Pseudoisochrysis paradoxa* than they did *Monochrysis lutheri*, although the two algal species are very similar in biochemical composition.

The food consumtion rate of juvenile oysters, as a percentage of their body weight per day, is inversely related to their size. Very small oysters (about .1 mg ash-free dry body weight) consumed up to 50 percent of their body weight per day at the same temperature. Literature reports indicate that adult bivalves (about 2.00 g dry body weight)consume only 1-3 percent of their body weight per day.

No growth was recorded among oysters held at temperatures above 19°C, regardless of the density of algae provided to them.

Increased growth possibly could be obtained at higher temperatures (above 20°C) if food quality could be improved.

Assimilation efficiency, a measure of the percentage of food consumed that is actually used by the animal for either respiration or growth, was relatively low among small oysters fed cultured algae. Assimilation efficiencies for very small

spat in our recirculating system ranged from 22 to 42 percent. Assimilation efficiency was inversely related to food density, but was not clearly related to temperature.

Maintenance requirements, the amount of food energy that must be assimilated just to maintain the animals' weight, was directly related to temperature. Measured assimilation, on the other hand, reached a maximum at 15°C to 19°C. Therefore, the difference between the amount of energy required just to maintain the animals and the amount of energy assimilated by the animals declined as temperatures exceeded at 15°C. Thus 15°C yielded the greatest growth.

RECOMMENDATIONS

Based on our studies, we conclude that the use of heated effluents for the intensive culture of the Pacific oyster is biologically feasible. However, our conclusion is based on the assumptions that the temperature in the culture system can be adjusted to about 15°C, and that some source of supplemental food for the oysters is available during the winter. We will now describe what a hypothetical culture system associated with a power plant might look like, and suggest additional research essential to the commercial success of such a system.

A Hypothetical Culture System

The system we describe is actually a composite of experimental systems, existing commercial culture systems, and a few completely untested ideas. We would like to provide a starting place, a first approximation, for the design of a culture system.

Obtaining oyster seed: For a variety of reasons outside the scope of this report, most of the commercial oyster hatcheries, the only reliable source of oyster seed, prefer to produce cultchless oyster seed. Moreover, cultchless seed offers the oyster farmer a number of advantages: light weight, ease of handling in intensive culture systems, and improved marketability because of its more uniform shape. The invisioned system therefore, is intended to handle only cutchless oysters obtained either from existing commercial hatcheries or from a hatchery that could be built as a part of the culture system itself.

Oyster rearing systems. Cultchless oysters must be held on trays or racks that allow use of all three dimensions of the water column, that prevent the oysters from washing together into piles in the corners of the tank or from being washed out of the tank completely, and that permit the oysters to be easily handled for sorting and washing. Different types of trays have been used for this purpose. One type that is available commercially and that seems to have rather widespread acceptance can be nested into stacks that are easy to handle and that permit circulation of water through the stack. However, this tray contains holes for water circulation that will not retain the smallest oyster spat, so that plastic window screen must be tied into the trays before they can be used for the early growth period. As soon as the spat are large enough to be retained in trays without screens, they should be transferred to trays that insure adequate movement of water, and should be sorted and thinned to permit rapid growth.

For the final growth period, the oyster farmer should build or purchase trays with a reasonably coarse mesh. Vinyl coated wire trays can be purchased, trays can be rather easily manufactured using a wooden or steel reinforcing rod frame covered with plastic netting. Netting is available in a variety of mesh sizes, to that a fine to medium mesh could be used for the intermediate growth period, and a coarse mesh could be used for the larger animals. Mesh size should be chosen to afford minimum waterflow restriction and yet retain the oysters. Since the oysters must be sorted and thinned periodically so that they will physically fit on the trays as they grow, they can also be transferred occasionally to a larger mesh size.

The design of the rearing tanks and water distribution system should create a well-distributed flow of water that removes waste materials and supplies each oyster in the system with water that has not been previously filtered free of food by other oysters.

Based on our own observations, we would recommend a tank design that creates an upward flow of water through stacked trays or racks of oysters. To provide an evenly distributed flow of water, the inflow to the tank should enter through slits running the length of each side of the tank at the bottom. The tank should be narrow and should include baffles that force the water to flow up through the stack of trays.

A traveling crane located above the tanks would assist in harvesting and could be used to lift the stacked trays out of the

tanks for washing in a tank filled with fresh water or a dilute chlorine solution. The fresh water or chlorine can help to control the growth of fouling organisms, many of which are not resistant to rapid reductions in salinity or to chlorine. Air drying or other chemical dips may also be used to control fouling organisms attached to the oysters or the trays (Hidu and Richmond 1974; Maurer and Aprill 1973).

Supplemental feeding. Although considerable work has been done during the last 30 years on development of an artificial food for oysters, at present no formulated diet exists that can be used as a substitute for natural food. Certain materials have been used successfully to "fatten" oysters--to induce a buildup of stored glycogen--but no studies have reported increases in both shell and meat among oysters fed solely on an artificial diet. Some of the more recent work used starch to increase the meat weight of mature *C. virginica* (Willis et al. 1976).

Obviously, a storable artificial food for oysters would revolutionize the oyster industry and would make large-scale intensive culture a reality. However, until an artificial diet is available, cultured algae will continue to be the only reliable material for supplementing the natural food of oysters and other bivalves. A great deal of research has been done recently to develop techniques for large-scale culturing of algae. A pilot plant using treated sewage as a fertilizer in algal culture was built at Woods Hole, Massachusetts (Ryther et al. 1973). Designed to provide a continuous supply of algae to cultured bivalves, this plant serves as a prototype for our hypothetical algal production facility.

Our studies have shown that young oysters grow quite well on a diet of the flagellate *Pseudoisochrysis paradoxa*. This species is also easy to culture, and specific information describing optimum culture conditions is available. Therefore, we have designed our hypothetical algae culture facility around the use of *P. paradoxa* produced in a system modified from those used in large experimental or commercial facilities.

In estimating our system's requirements for cultured algae, we will assume a number of constants to be valid. However, we emphasize, that a number of factors will influence these "constants." Factors such as site location and season will of course influence the quantity and quality of natural food available to the oysters, which will in turn affect the need for supplemental food. Other factors such as nutrient source and concentration, temperature, culture density, and light intensity will influence the weight and the nutritional value of the cultured algae cells. With these cautions in mind, we suggest the following.

1. A desirable level of particulate organic carbon provided to the oysters is about 700 µg C/L (see Fig. 3); we assume a background of 200 µg C/L, so we propose to supplement at a rate of 500 µg C/L entering the oyster growing tanks.

2. Each *Pseudoisochrysis paradoxa* cell contains approximately 5.2×10^{-6} g of carbon (Malouf 1977); 500 µg C/L is, therefore, about 100,000 algal cells/ml.

3. Our culture system would contain 10×10^6 juvenile oysters, and we would supply a water flow rate of 10 ml/oyster/min, or a total flow of 10×10^4 l/min.

4. Maintaining the desired algal cell concentration at that flow rate would require 10×10^{12} algae cells/min, or 14×10^{15} cells/day.

5. We will assume that the algal culture ponds will reach a density of 3×10^6 cells/ml (3×10^9 cells/l); therefore, we would require 4.7×10^6 l/day from the algae cultures.

6. Our primary culture ponds--concrete basins 2 m deep--would have .6 ha of surface area each and would be filled to a depth of 1.33 m. Since .6 ha equals 6070 m^2 at a depth of 1.3 m, the ponds would contain 7.9×10^6 l each.

7. We expect to harvest 33 percent of the algae culture volume each day and to refill the pond with seawater and fertilizer (Ryther et al. 1973). Thus, each primary pond could supply 2.6×10^6 l/day, and two such ponds could supply our daily requirement for algae (4.7×10^6 l/day).

8. The system should include four primary ponds. Two will be harvested daily for at least two weeks before we would expect contamination with undesirable algal species; one other will have been inoculated with algae and will have increasing algal density, and the fourth will be ready for cleaning and sterilization. This cycle would repeat approximately every two to four weeks.

9. The four ponds should be physically isolated from each other as much as possible.

Older algae cultures in open ponds inevitably become contaminated, and isolation will help to prevent cross-contamination between the older cultures and the freshly inoculated ponds.

10. An inoculum of algae equal to about 5 percent of the primary ponds' volume should be used to start these large cultures. This will require four secondary culture ponds containing 40 x 10^4 l each. These should be no more than 1 m deep, with dimensions approximately 10 m x 40 m.

11. Secondary ponds would also require an inoculum equal to about 5 percent of their volume. This would call for tertiary cultures of about 20,000 algal cells per ml. Ideally, there should be two of these for each secondary culture, since this is the largest size at which backup cultures may be practical. Tertiary cultures may be about .5 m deep and 4 m x 10 m, and might also be covered by plastic greenhouses (two tanks per greenhouse) approximately 20 m long and 6 m wide.

12. The tertiary algae culture ponds should be inoculated from 1000 l culture contained in a laboratory support building. These 1000 l cultures would in turn be inoculated from 50 l tanks maintained from sterile stock cultures as described by Breese and Malouf (1975).

13. All of the cultures 1000 l or less in volume would use seawater sterilized by chlorination-carbon filtration or by autoclaving. All would be lighted by fluorescent bulbs.

14. Seawater used in the primary, secondary, and tertiary cultures, 5-6 x 10^6 l/day, must be sand filtered to at least 5 μm. Heat from the power plant or high-volume ultraviolet sterilizers might also be used to sterilize this water. If the required water was pumped 24 hours a day, which is likely, a flow of 4170 lpm (1100 gpm) would be required.

We have outlined in detail the type of algae production system that a large-scale intensive oyster culture might require. Our data and the work of others (Price et al. 1976) show conclusively that some supplemental feeding would be essential if intensive oyster culture associated with higher than ambient temperatures is to be biologically feasible (see Fig. 3). Our estimates of the algae production facilities well include some error, but should show the scale of these facilities with reasonable accuracy. Obviously, 2 ha of algae culture ponds represent considerable capital investment, perhaps more than the oyster growing tanks. In any case, the development and production of a storable artificial food for oysters clearly would reduce the scale of the total culture system by eliminating the need for algae culture, and would contribute immeasurably both to the reliability of the system and to its production potential.

Some additional considerations. We have dealt with some of the problems inherent in the use of heated water from power generating plants for culturing oysters. However, we have not discussed a number of additional problem areas. Most of these are associated with the power plant itself and were beyond the scope of our experimental work. In the following section, we will briefly discuss some of the work of other researchers and consider critical areas requiring additional study.

Seawater's passage through the cooling system of a power generating plant can put the seawater in contact with metal components of that system, increase the concentrations of heavy metal ions in seawater exiting the power plant. Although concentrations of heavy metals would probably not in themselves cause mortality among oysters cultured in the plant's effluent, oysters can concentrate these heavy metals in their tissues (Galtsoff 1964). Abnormal metal content in the oyster meat could create a health hazard for the consumer or affect the product's marketability. Studies indicate that copper is one of the metals that accumulates in oysters held in power plant effluents (Roosenburg 1969; Gilmore et al. 1975). Zinc can also cause problems in some situations (Wolfe 1970). The nature and degree of the heavy metal problem depends on the design of the plant, but the designers of culture systems using power plant effluents must not overlook the problem.

Bivalve mullusks, including oysters, are able to accumulate radioactive materials in their environment (Price 1962; Wolfe 1970). Rates of accumulation of these materials depend upon the chemical and physical properties of the radiosotopes and upon the organisms' interactions with their environment. Temperature, salinity, and pH may influence the rates of uptake and levels of accumulation of certain isotopes, particularly those that are of metabolic significance to the animal (Wolfe and Coburn 1970). This is potentially critical to the economic success of any oyster culture system associated with a nuclear power plant

(Price et al. 1976).

Chlorine is widely used for control of fouling organisms in the cooling systems of electric power generating plants, and adversely affects the productivity of algae contained in the water (Carpenter et al. 1972). The use of chlorine could diminish the quality of natural food available to oysters held in a plant's effluents, and also the feasibility of using the effluents in mass algae culture.

Aquaculturists who have attempted to use the effluents from power plants face difficulties when the plants undergo one of the periodic shutdowns (Anon. 1977). Oysters can survive long periods in reduced water flow of water, and, compared to fish, they are resistant to reduced oxygen concentrations and rapid temperature changes. However, their feeding and growth would be adversely affected by lengthy shutdowns. Some alternative system for providing water flow would be required if interruptions in the power plants' effluents were expected to occur with any frequency.

We have briefly alluded to the problem of controlling disease among oysters intensively cultured at increased temperatures. The Pacific oyster is susceptible to bacterial diseases that may become critical under crowded conditions silmultaneously providing relatively high concentrations of organic materials in warm seawater (Lipovsky and Chew 1973; Grischkowsky and Liston 1974). Control of bacterial and other diseases in an intensive culture system would be essential. Currently, techniques for disease control (ultraviolet treatment of the water supply, use of antibiotics, and other methods) have proven only partially successful. Moreover, the use of antibiotics or chemicals in food culture is restricted by the Food and Drug Administration, so that disease control techniques promising on an experimental basis may not be applicable at present to commercial culture systems.

Solution of most of these problems, and indeed the successful operation of an intensive culture system in general, would require close cooperation between the aquaculturists and operators of the power generating plant. An intensive oyster culture facility, or any other type of culture facility, could not possibly succeed in the effluent of a power plant unless the operators of the plant understood aquaculturists' absolute requirement for a relatively stable environment free from harmful substances.

Clearly, additional information on all aspects of the intensive culture of Pacific oysters would be helpful in the final design of a culture facility. However, feeding and nutrition and disease control are two specific problem areas in which there is a critical need for additional research.

Without significantly improved understanding of the nutritional requirements of oysters, culminated eventually by the development of an inexpensive artificial food, the economic feasibility of intensive oyster culture must remain in doubt. The massive investment required to produce living food (algae) for oysters should be painfully obvious from our discussion, but the unreliable nature of algae culture may not be so evident. Experience has shown that it is actually more difficult to provide optimum conditions for algae growth than for oysters. Elimination of the need for algae culture would relieve the oyster farmer of a significant economic burden, and, equally important, would increase the total system's reliability.

Finally, we would like to emphasize that our knowledge of techniques for disease control in intensively cultured Pacific oysters is limited at best. At present the only universally accepted means of controlling such diseases is through culture conditions that inhibit the growth of the organism. Unfortunately, this is likely to inhibit concurrently the growth of the oysters, since it generally requires low temperatures and low densities of organic materials. Positive disease controls must be developed and accepted by the Food and Drug Administration and other regulatory agencies before full advantage can be taken of the food producing potential of heated power plant effluents.

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