AN ABSTRACT OF THE THESIS OF

MARGARET ANN TONER for the degree of MASTER OF SCIENCE

in FISHERIES AND WILDLIFE presented on June 26, 1978

Title: COMPARATIVE GROWTH OF JUVENILE PACIFIC OYSTERS (CRASSOSTREA GIGAS) FED UNIALGAL VS. SYNTHESIZED DIETS

Abstract approved: Redacted for Privacy
Howard F. Horton

The results of research designed to develop a synthesized diet as a substitute or supplement to algae for culturing the Miyagi strain of juvenile Pacific oysters, *Crassostrea gigas* (Thunberg) are reported. The growth of juvenile Pacific oysters fed equal amounts (based on ash free dry weights [AFDW]) of algae (*Pseudoisochrysis paradoxa*, lysed algae, dried oyster meat, and encapsulated lysed algae was determined in five experiments. Dried oyster meat and encapsulated lysed algae were also fed as supplements to lysed algae at three levels: 25, 50 and 75 percent of the total AFDW of the diet. In addition, the growth of oysters fed lysed and whole algal cells with and without the addition of sodium sulfamethazine (Sulmet) was compared. Growth was measured as the difference in AFDW and in shell length between treatments and an initial sample frozen at the beginning of the experiment.

In the first experiment, oysters fed algae alone showed an increase in AFDW and in shell length that was significantly greater than that of oysters fed dried oyster meat alone and as a supplement to algae. In the second experiment, oysters fed lysed algae treated with or without Sulmet showed a significant decrease in AFDW from the initial sample. Oysters fed algae with and without treatment with Sulmet did not show a significant increase in AFDW. No shell growth was observed in the remaining experiments. In the third experiment, oysters fed starch encapsulated algae and algae with and without supplementation with encapsulated algae increased significantly in AFDW as compared to the initial sample. In the fourth and fifth experiments, oysters fed starch encapsulated algae only did not show a significantly greater AFDW than the initial sample, but these oysters did not lose weight during the experiments.

Encapsulation appears to be a process which could be used as a delivery system for feeding synthesized diets to juvenile oysters.

# Comparative Growth of Juvenile Pacific Oysters (*Crassostrea gigas*) Fed Unialgal vs. Synthesized Diets

by

Margaret Ann Toner

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Completed June 1978

Commencement June 1979

APPROVED:

Redacted for Privacy

Professor of Fisheries

in charge of major

Redacted for Privacy

Head of Department of Fisheries and Wildlife

Redacted for Privacy

Dean of Graduate School

Date thesis is presented June 26, 1978

Typed by Clover Redfern for Margaret Ann Toner

## ACKNOWLEDGMENT

I would like to thank Dr. Duncan Law of the Oregon State University Seafoods Laboratory, Astoria, Oregon for his assistance in formulating the ground oyster meat diet and the encapsulated diet. I am also indebted to Dr. Robert Malouf for his advise on the design for my experiments.

I would also like to thank Prof. R. Sinnhuber for his advise on the formulation of diets and Dr. J. Lannan for his support and advise while I was conducting my experiments. Special thanks go to my major professor, Dr. Howard Horton, for helping me with this thesis. Finally, I am grateful to Prof. W.P. Breese for helping me to obtain experimental animals and research apparatus, and for supplying funding through the Sea Grant College Program, which supported my research under Sea Grant Project No. R/AQ-26.

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# COMPARATIVE GROWTH OF JUVENILE PACIFIC OYSTERS (CRASSOSTREA GIGAS) FED UNIALGAL VS. SYNTHESIZED DIETS

## INTRODUCTION

The purpose of the following study was to investigate the development of synthesized diets as a substitute or supplement to algae for feeding juvenile Pacific oysters, Crassostrea gigas, in oyster hatcheries. The experiments were conducted from 18 Jul to 19 Dec 76 and from 1 Jul to 21 Oct 77 in conjunction with the experimental oyster hatchery of the Oregon State University Marine Science Center, Newport, Oregon. Synthesized diets that could be stored would have many advantages over the use of algae in culturing bivalves because of the space required for culturing large volumes of algae and because of the time invested in maintenance of the algae cultures. The synthsized diets in the following studies were fed singly and as supplements to the alga Pseudoisochrysis paradoxa (VA-12) in ratios of 25:75, 50:50, and 75:25. The diets consisted of dried, ground oyster meat and a microencapsulated food. Another diet tested was a dissolved organic diet derived from lysed algae.

The nutritional requirements and food preferences of oysters have not been clearly established. Early food habit studies involved examining the contents of the digestive tract to establish their nutritional needs (Moore, 1908; Savage, 1925). This approach is subject to

criticism because the contents indicate the type of available particles and not their nutritional value to the oyster. The potential food sources for oysters include phytoplankton, suspended dead organic matter or detritus, dissolved organic matter, heterotrophic organisms such as bacteria and yeasts, and heterotrophic flagellates.

Detritus has been found as a component of the diet of oysters. Korringa (1949) reported that detritus from animal and plant material provided normal growth in oysters. Jensen (1915) attempted to show that detritus derived from Zostera marina was a component of the feces of oysters. Blegvad (1915) suggested that bivalves were detritus feeders.

Matthiessen and Toner (1966) did not obtain any growth in oysters fed detritus prepared from wheat germ, Pablum, Brewer's yeast, non-fat dry milk, Zostera marina, and Fucus vesiculosus. Ingle (1967) did not observe growth of oysters fed powdered milk and a substance high in amino acid content, but did observe an increase in total weight and an accumulation in food reserves of oysters fed corn meal.

The condition indexes of Pacific oysters were increased by supplementing unfiltered seawater with corn starch (Sayce and Tufts, 1967). Haven (1965) reported that American oysters (Crassostrea virginica) held in seawater supplemented with corn or wheat starch increased in tissue weights, but not in shell size. Gillespie et al.

(1967) found that the glycogen content and dry weight increased when oysters were fed finely ground cornmeal.

Diets were formulated by Biddle at the University of Delaware using protein levels at 20 and 40% of the dry weight of the diet and using three coating agents (Epifanio, 1975). The diets were micronized to a mean particle size of 11 μm and fed to larval clams (*Mercenaria mercenaria*) and to juvenile clams and oysters (*C. virginica*). The larval clams grew more rapidly than the unfed controls when fed the test diets, but not as rapidly as the larvae fed *Isochrysis galbana*. The juvenile oysters and clams fed the test diets did not show significant shell growth after four weeks.

Castell and Trider (1974) synthesized diets for oysters in which the levels of lipid and carbohydrate were varied and found that increased levels of diet carbohydrate and higher levels of diet lipid yielded a more rapid initial weight gain. Dunathan et al. (1969) observed an increase in oyster meat glycogen content when higher levels of carbohydrate were fed. Finely ground cornmeal and brown rice produced the highest glycogen accumulation in adult oysters of all of the cereals tested (Dunathan et al., 1969). Kern (1973) reported that juvenile oysters fed algae supplemented with rice starch exhibited growth equal to that shown by oysters fed algae alone when the oysters were fed the diets on an equal caloric value basis.

Bacteria have produced a growth response in some bivalves, i.e., *Mytilus californianus* (Zobell and Feltham, 1937), but Davis (1953) observed no growth when 13 species of marine bacteria were fed to oyster larvae. Guillard (1959) attributed larval mortality to a few species of bacteria.

Dissolved organic materials appear to be utilized as food by bivalves. Yonge (1928) found oysters were able to utilize glucose under abnormal conditions by absorption by phagocytes. Mitchell (1915) found an increase in the glycogen content of oysters that were held in a glucose solution for several days. DeBoer (1975) reported that dissolved organic materials and organic aggregates were incorporated by oyster larvae. Collier (1959) found oysters removed 5 to 15% of dissolved carbohydrate from water.

It has been demonstrated that certain species of phytoplankton produce growth in bivalves. Davis and Guillard (1958) found *Isochrysis galbana* and *Monochrysis lutheri* were suitable foods for oyster larvae. Cole (1937) found the green flagellate *Tetraselmis* supported the growth of oyster larvae. The abundance of *Skeletonema costatum* in Delaware Bay was the attributed cause of oyster fattening (Nelson, 1947). *Cylindrotheca gracilis* and *Navicula abscondita* were reported as adequate food sources for oyster larvae by DeMort (1970). Walne (1970) showed that *Isochrysis galbana* supported good growth of *C. gigas* juveniles. Ryther and Goldman (1975) showed that

*Thalassiosira pseudonana* was a successful larval food.

As yet it is not known why particular algal species are superior to other species as food for culturing oysters. Walne (1970) suggested that algae with rigid cell walls such as *Chlorella* and *Chlamydomonas* are not readily assimilated by juvenile bivalves, but this does not account for an alga such as *Dunaliella* which has no rigid cell wall, and which does not produce good growth in bivalves either. DeMort (1975) could not account for the differences in nutritional value of estuarine phytoplankton species fed to oyster larvae based on the amino acid or fatty acid contents.

There is evidence that a single species of algae does not produce optimum growth when fed to bivalves. Davis and Guillard (1958) found that feeding bivalve larvae mixtures of different species of algae produced greater growth than feeding one algal species individually. Malouf (1977) found that juvenile oysters held in unfiltered seawater showed very good growth, although in caloric terms, the seawater contained a relatively small amount of food.

It is possible that vitamins help determine the nutritional value of phytoplankton to oysters. Kern (1973) found that juvenile oysters fed algae supplemented with vitamins grew significantly faster than oysters fed unsupplemented algae.

# METHODS

## Seawater

Seawater was obtained from Yaquina Bay through the seawater supply of the Oregon State University Marine Science Center, Newport, Oregon. Water was obtained at high tide when salinity ranged from 30-33 ppt. Water was passed successively through a settling basin, sand filters (Northwest Filter Co., Inc.), an ultraviolet light sterilization unit (AquaNomics, Inc.), a 3.0 μm cartridge filter (Pall, Epocel-3) and a 0.8 μm cartridge filter (Pall, Ultipor-.25). Water was then stored 12-20 hr in the dark in either covered plastic 121-liter containers (Experiments 1, 2 and 3) or a covered 150-liter fiberglass container (Experiments 4 and 5).

## Oysters

Cultchless, juvenile Pacific oysters (*Crassostrea gigas*) of the Miyagi strain were obtained from the Pigeon Point Shellfish Hatchery, Pescadero, California and held in running, unfiltered seawater for one month. Oysters with new shell growth were selected for experimentation. An attempt was made to select oysters of the same size and to exclude the smaller and the larger animals.

Growth of oysters was determined using methods similar to those described by Malouf (1977). Shell size was determined by

measuring oysters with a micrometer eyepiece to the nearest 0.1 mm. The longest dimension of the shell, referred to as length, was measured at the beginning and the end of each experiment.

Ash free dry weight (AFDW) of oyster meats was determined by first shucking the oysters with concentrated hydrochloric acid to dissolve the shell (Malouf, 1977). The calcium chloride salts formed by the reaction of the acid and the calcium carbonate of the shell were removed from the meats by rinsing the oysters for 10 min in glass-distilled water. The rinsed oyster meats were then placed on glass fiber filters (Whatman GF/A) that had been ashed at 450 C and weighed. Next, the meats were dried for 20 hr at 90 C and weighed to the nearest μg on an electrobalance to obtain a measure of dry weight. Finally, the meats were ashed for 20 hr at 450 C, the ash was rehydrated with glass-distilled water, and the rehydrated ash was dried for 20 hr at 90C and weighed to the nearest μg. The weight of the ash subtracted from the weight of the dry meat gave the AFDW.

A sample of oysters was taken at the beginning of each experiment to provide a measure of the initial shell height and length, and the initial AFDW of the meat. Growth was then determined as the change in shell dimensions and AFDW of meats when the mean values of initial and treated samples were compared.

## Algae

The alga Pseudoisochrysis paradoxa (VA 12), referred to in this thesis simply as algae, was used as a standard for comparison when feeding test diets, as this alga is known to be an adequate food for oysters (Malouf, 1977). Algae was obtained from Dr. John Dupuy of the Virginia Institute of Marine Science. The system used to culture algae was that of the Oregon State University experimental oyster hatchery described by Malouf (1977).

Fifty-liter algae cultures were harvested during the log growth phase when cell counts were approximately 8-10 million cells/ml. Algal cell counts were made with a Coulter Counter Model ZBI fitted with a 70 µm aperture tube.

## Preparation of Diet Materials

To prepare the diet for the first experiment, shucked adult oysters were blended with 1% agar (by dry weight) and the mixture was dried with a drum drier at the Oregon State University Seafoods Laboratory, Astoria, Oregon. The dried oyster meat was ground with a rolling jar mill until the desired particle size of 5-20 µm diameter was reached. The diet was weighed on an analytical balance and was suspended in 30 ml of filtered seawater by mixing for 30 sec with a Tissumizer Model SDT-182N (Tekmar Co.) before being fed to the oysters.

To prepare the diet for the second experiment, the algal cells were lysed with a Biosonic III sonicator (Bronwill Scientific) for 6 min, which left approximately 1% of the cells intact. The bacteriostat sodium sulfamethazine (Sulmet) was used to determine whether bacterial activity influenced growth at a dosage of 50 ppm as recommended by Hidu and Ukeles (1962). The lysed and whole algal cells were fed to oysters alone and with the addition of Sulmet.

To prepare the diets for the third, fourth, and fifth experiments, a culture of algae was centrifuged in a Servall superspeed centrifuge (St. Gyorgyi and Blum Continuous Flow System) at 50% of the maximum output voltage. The Coulter Counter was used to determine the number of cells in the cultures before centrifuging and the number of cells in the outflow in order to estimate the number of cells recovered.

The centrifuge pellet was suspended in distilled water and was lysed for 6 min with a Biosonic III sonicator. An antioxidant solution composed of 3 g butylated hydroxytoluene (BHT), 3 g butylated hydroxyanisole (BHA), 54 g propylene glycol and 49 g of Tween 20 (Eastman Kodak) was added to the lysed algae at a concentration of 0.1% of the lipid content of the algae (unpublished report, May 3, 1976, from R.O. Sinnhuber, Dept. of Food Science and Technology, Oregon State University). Amaizo W-11 starch (American Maize Products Co.) was mixed with distilled water and retorted to dissolve the starch. After retorting, the starch solution was cooled to 45 C

and blended with the lysed algae at a ratio of 80:20 (w/w). The mixture was agitated with a magnetic stirrer and spray dried in a Niro Atomizer with an internal temperature of 220 C and an outlet temperature of 100-120 C.

In the third experiment, this procedure was repeated with the substitution of Amaizo Amylomaize VII starch (American Maize Products Co. ) for the Amaizo W-11 starch. The lysed algae alone was also spray-dried.

In the fourth and fifth experiments, only the Amaizo W-11 starch was blended with the lysed algae. The retorted Amaizo W-11 starch was also spray dried alone. The dried materials were weighed and suspended before being fed using the same procedure as that described for the dried oyster diet.

## Experimental Procedure

A static, closed water system was used in all of the experiments. Experiments were conducted in 10-liter polypropylene buckets (Rubbermaid Commercial Products, Inc. ). Fifty oysters were held on a piece of Nytex screen cut to fit in the bottom of each container. The Nytex screen allowed the oysters to be handled more easily during water changes. Every day the oysters were removed and were rinsed with dechlorinated water. The containers were

emptied, washed with dechlorinated water, and were filled with fresh filtered seawater.

Experiments 1, 2, and 3 were conducted in a room in which the water temperature varied from 12.2-20.5 C. Experiment 1 was conducted from 18 Jul to 5 Sep 76; Experiment 2 was conducted from 14 Sep to 8 Nov 76 and Experiment 3 was conducted from 8 Nov to 19 Dec 76.

Experiments 4 and 5 were conducted in a constant temperature room in which the water temperature varied from 15 to 17 C. Experiment 4 was conducted from 1 Jul to 26 Aug 77 and Experiment 5 was conducted from 26 Aug to 21 Oct 77.

Based on their AFDW, equal amounts of the algae and the test diets were fed to the oysters. Each treatment was applied to two containers in all of the experiments. Ingestion of the diets by the oysters was confirmed by examining samples of the feces after the oysters were fed the diets to determine whether intact diet particles were identifiable. The treatments of Experiments 1, 2 and 3 are given in Tables 1, 2 and 3 respectively.

In Experiments 4 and 5, the containers were aerated with micropipettes which allowed a flow of very fine bubbles to prevent stratification. For two weeks each of the containers received $1 \times 10^9$ cells of algae daily. At the end of two weeks, an initial sample of approximately 100 oysters was frozen. Then the test diets for Experiments

4 and 5, as shown in Tables 4 and 5 respectively, were fed for eight weeks.

## Statistical Treatment

The lengths and weights of oysters in all experiments were assumed to be normally distributed. The Statistical Package for the Social Sciences (SPSS) computer program was used to compute an analysis of variance. The least significant difference (LSD) was computed for each treatment to determine whether the sample means were different at the 0.05 level of significance.

Table 1. Design of Experiment 1 indicating the treatments and quantity of diet components fed to juvenile Pacific oysters (*Crassostrea gigas*) daily.

| Treatment | Diet Components Fed Daily | | | |
|---|---|---|---|---|
| | Algal[a] Cells (x $10^8$) | Algal AFDW[b] (mg) | Dried Oyster AFDW (mg) | Agar Binder AFDW (mg) |
| Initial | -- | -- | -- | -- |
| Unfed control | -- | -- | -- | -- |
| Algae | 10.0 | 17.5 | -- | -- |
| Dried oyster | -- | -- | 17.5 | -- |
| Oyster:algae 75:25 | 2.5 | 4.4 | 13.1 | -- |
| Oyster:algae 50:50 | 5.0 | 8.8 | 8.8 | -- |
| Oyster:algae 25:75 | 7.5 | 13.1 | 4.4 | -- |
| Agar binder | -- | -- | -- | 17.5 |

[a] *Pseudoisochrysis paradoxa* (VA-12).

[b] Ash free dry weight.

Table 2. Design of Experiment 2 indicating the treatments and quantity of diet components fed to juvenile Pacific oysters (*Crassostrea gigas*) daily.

| Treatment | Diet Components Fed Daily | | |
|---|---|---|---|
| | Algal[a] Cells ($x 10^8$) | Algal AFDW[b] (mg) | Dried Oyster AFDW (mg) |
| Initial | -- | -- | -- |
| Unfed control | -- | -- | -- |
| Algae | 10.0 | 17.5 | -- |
| Algae + Sulmet[c] | 10.0 | 17.5 | -- |
| Lysed algae | 10.0 | 17.5 | -- |
| Lysed algae + Sulmet | 10.0 | 17.5 | -- |
| Dried oyster | -- | -- | 175.0 |

[a] *Pseudoisochrysis paradoxa* (VA-12).

[b] Ash free dry weight.

[c] Fed at rate of 0.5 g sodium sulfamethazine (Sulmet) per experimental group.

Table 3. Design of Experiment 3 indicating the treatments and quantity of diet components fed to juvenile Pacific oysters (Crassostrea gigas) daily.

| Treatment | Diet Components Fed Daily | | |
|---|---|---|---|
| | Algal[a] Cells (x $10^8$) | Algal AFDW[b] (mg) | Starch[c] or Capsules AFDW (mg) |
| Initial | -- | -- | -- |
| Unfed control | -- | -- | -- |
| Algae | 10.0 | 17.5 | -- |
| Algae - dried | -- | 17.5 | -- |
| Lysed algae - dried | -- | 17.5 | -- |
| Starch (W-11) encapsulated algae | -- | -- | 17.5 |
| Starch (VII) encapsulated algae | -- | -- | 17.5 |
| W-11 capsules:algae 25:75 | 7.5 | 13.1 | 4.4 |
| W-11 capsules:algae 50:50 | 5.0 | 8.8 | 8.8 |
| W-11 capsules:algae 75:25 | 2.5 | 4.4 | 13.1 |
| Starch (W-11) | -- | -- | 17.5 |
| Starch (VII) | -- | -- | 17.5 |

[a]Pseudoisochrysis paradoxa (VA-12).

[b]Ash free dry weight.

[c]Amaizo W-11 and Amaizo VII Starch (American Maize Product Co.).

Table 4. Design of Experiment 4 indicating the treatments and quantity of diet components fed to juvenile Pacific oysters (Crassostrea gigas) daily.

| Treatment | Diet Components Fed Daily | | |
|---|---|---|---|
| | Algal[a] Cells (x $10^8$) | Algal AFDW[b] (mg) | Starch[c] or Capsules AFDW (mg) |
| Initial | -- | -- | -- |
| Unfed control | -- | -- | -- |
| Algae | 10.0 | 17.5 | -- |
| Encapsulated algae | -- | -- | 17.5 |
| Capsules:algae 25:75 | 7.5 | 13.1 | 4.4 |
| Capsules:algae 50:50 | 5.0 | 8.8 | 8.8 |
| Capsules:algae 75:25 | 2.5 | 4.4 | 13.1 |
| Starch | -- | -- | 17.5 |

[a] Pseudoisochrysis paradoxa (VA-12).

[b] Ash free dry weight.

[c] Amaizo W-11 Starch (American Maize Products Co.).

Table 5. Design of Experiment 5 indicating the treatments and quantity of diet components fed to juvenile Pacific oysters (*Crassostrea gigas*) daily.

| Treatment | Diet Components Fed Daily | | |
|---|---|---|---|
| | Algal[a] Cells (x $10^8$) | Algal AFDW[b] (mg) | Starch[c] or Capsules AFDW (mg) |
| Initial | -- | -- | -- |
| Unfed control | -- | -- | -- |
| Algae | 10.0 | 17.5 | -- |
| Encapsulated algae | -- | -- | 17.5 |
| Capsules:algae 25:75 | 7.5 | 13.1 | 4.4 |
| Capsules:algae 50:50 | 5.0 | 8.8 | 8.8 |
| Capsules:algae 75:25 | 2.5 | 4.4 | 4.4 |
| Starch | -- | -- | 17.5 |
| Capsules + Sulmet[d] | -- | -- | 17.5 |

[a] *Pseudoisochrysis paradoxa* (VA-12).

[b] Ash free dry weight.

[c] Amaizo W-11 Starch (American Maize Products Co.).

[d] Using at the rate of 0.5 g sodium sulfamethazine (Sulmet) per experimental group.

# RESULTS

## Experiment 1

Oysters fed algae gained a significant amount of AFDW as well as increasing shell length (Tables 6 and 7). The algae fed group showed the largest amount of AFDW and the greatest increase in shell length of any of the treatments. There was no significant difference in the AFDW among the groups of oysters fed the dried oyster alone or as a supplement to algae (Table 6). The oysters fed algae combined with dried oyster meat appeared to have increased shell length slightly, while the oysters fed dried oyster meat only showed no shell growth (Table 7). The oysters fed ground agar that was used as a binder in the ground, dried oyster diet lost a significant amount of AFDW (Table 6). The average shell length for the agar fed treatment was significantly less than that of the initial sample at the end of the experiment, while it had not been significantly different at the beginning (Table 7). There was no significant difference between the AFDW or the average length of the initial and unfed treatments (Tables 6 and 7).

## Experiment 2

No shell growth was shown by any of the treatments in Experiment 2 (Table 8) with the exception of those fed oyster meat where the

Table 6. Average (N = 100) initial ash free dry weight (AFDW) for juvenile Pacific oysters and after treatment with seven feeding regimes (Experiment 1).

| Treatment | AFDW (mg) |
|---|---|
| Agar | 0.268[a] |
| Initial | 0.385[b] |
| Unfed control | 0.400[b] |
| Oyster:algae[f] 75:25 | 0.526[a, c] |
| Oyster | 0.577[a, c, d] |
| Oyster:algae 25:75 | 0.651[a, d, e] |
| Oyster:algae 50:50 | 0.696[a, e] |
| Algae | 1.093[a] |

[a]Significantly different from initial weight at 95% level.

[b, c, d, e]No significant difference at 95% level.

[f]Pseudoisochrysis paradoxa (VA-12).

Table 7. Average (N = 100) length for juvenile Pacific oysters at beginning and end of seven feeding regimes (Experiment 1).

| Treatment | Length (mm) | | |
|---|---|---|---|
| | Beginning | End | Difference |
| Initial | 6.9[d, e] | -[c] | - |
| Unfed control | 6.6[c, d] | 6.9[c] | +0.3 |
| Algae[a] | 7.9[b] | 9.2[b] | +1.3 |
| Oyster | 7.5[b, f] | 7.4[b, d] | -0.1 |
| Oyster:algae 25:75 | 7.4[b, f] | 7.8[b, e] | +0.4 |
| Oyster:algae 50:50 | 7.0[e] | 7.6[b, d, e] | +0.6 |
| Oyster:algae 75:25 | 6.5[b, c] | 7.3[b, d] | +0.8 |
| Agar binder | 6.9[d] | 6.5[b] | -0.4 |

[a] *Pseudoisochrysis paradoxa* (VA-12).

[b] Significantly different from initial length at 95% level.

[c, d, e, f] No significant difference at 95% level.

Table 8. Average (N = 100) length of juvenile Pacific oysters on six feeding regimes (Experiment 2).

| Treatment | Length (mm) | | |
|---|---|---|---|
| | Beginning | End | Difference |
| Initial | 4.5[e] | -[e] | - |
| Unfed control | 4.6[e] | 4.5[e] | -0.1 |
| Lysed algae[a] | 4.1[c, d] | 4.0[c] | -0.1 |
| Lysed algae + Sulmet[b] | 4.2[c, d] | 4.2[c, d] | - |
| Algae | 4.8[c] | 4.6[e] | -0.2 |
| Algae + Sulmet | 5.0 | 4.9[c] | -0.1 |
| Oyster | 4.2[c, d] | 4.3[c, d] | +0.1 |

[a] Algae used was *Pseudoisochrysis paradoxa* (VA-12).

[b] Sodium sulfamethazine.

[c] Significantly different from the initial length at the 95% level.

[d, e] No significant difference at 95% level.

apparent gain of 0.1 mm is attributed to rounding error. Oysters fed lysed algae with and without the addition of the bacteriostat Sulmet and the unfed control lost a significant amount of AFDW as compared to the initial sample (Table 9). The AFDW of the remaining treatments were not significantly greater than that of the initial sample. There was no significant difference between the lengths of the initial and unfed oyster samples (Tables 8 and 9).

## Experiment 3

There was no significant difference among the AFDW of oysters fed algae and oysters fed encapsulated algae alone or as a supplement to algae, but these treatments produced AFDW greater than those of the initial or unfed samples (Table 10). There was no significant difference among the AFDW of the unfed oysters and the oysters fed dried whole algae or the starches used to encapsulate the algae (Table 10). No appreciable shell growth was shown by any of the treatments in Experiment 3 (Table 11). The length of the initial sample was significantly smaller than those of the other treatments, but the AFDW was not significantly different from that of the unfed sample (Table 10).

## Experiment 4

The AFDW of the oysters fed encapsulated algae alone was significantly different from that of the initial sample (Table 12). The

Table 9. Average (N = 100) initial ash free dry weight (AFDW) for juvenile Pacific oysters and after treatment with six feeding regimes (Experiment 2).

| Treatment | AFDW (mg) |
|---|---|
| Lysed algae[a] | 0.056[b, c] |
| Lysed algae + Sulmet[e] | 0.064[b, c] |
| Unfed | 0.084[b, c] |
| Algae | 0.143[d] |
| Initial | 0.144[d] |
| Algae + Sulmet | 0.149[d] |
| Oyster | 0.151[d] |

[a]Algae used was *Pseudoisochrysis paradoxa* (VA-12).

[b]Significantly different from initial weight at 95% level.

[c, d]No significant difference at 95% level.

[e]Sodium sulfamethazine.

Table 10. Average (N = 100) initial ash free dry weight (AFDW) for juvenile Pacific oysters and after treatment with 11 feeding regimes (Experiment 3).

| Treatment | AFDW (mg) |
|---|---|
| Initial | 0.106[d] |
| Unfed | 0.130[d, e] |
| Dried algae[a] | 0.140[e, f] |
| Starch (VII)[b] | 0.141[e, f] |
| Starch (W-11)[b] | 0.157[e, f, g] |
| Lysed algae - dried | 0.168[c, f, g, h] |
| Algae | 0.186[c, g, h, i] |
| Starch (VII) encapsulated algae | 0.186[c, g, h, i] |
| W-11 capsules:algae 50:50 | 0.191[c, h, i] |
| W-11 capsules:algae 25:75 | 0.199[c, h, i] |
| Starch (W-11) encapsulated algae | 0.218[c, i, j] |
| W-11 capsules:algae 75:25 | 0.243[c, j] |

[a]Algae used was Pseudoisochrysis paradoxa (VA-12).

[b]Amaizo VII and W-11 Starch (American Maize Products Co.).

[c]Significantly different from the initial value at the 95% level.

[d, e, f, g, h, i, j]No significant difference at the 95% level.

Table 11. Average (N = 100) length for juvenile Pacific oysters on 11 feeding regimes (Experiment 3).

| Treatment | Length (mm) | | |
|---|---|---|---|
| | Beginning | End | Difference |
| Starch (W-11)[a] | 5.3 | 5.1 | -0.2 |
| Starch (VII)[a] | 4.9 | 4.8 | -0.1 |
| Dried algae[b] | 4.6 | 4.6 | - |
| Lysed algae - dried | 4.9 | 4.9 | - |
| Algae | 5.0 | 5.3 | +0.3 |
| W-11 encapsulated algae | 5.0 | 5.0 | - |
| VII encapsulated algae | 4.8 | 5.0 | +0.2 |
| W-11 capsules:algae 25:75 | 5.0 | 5.0 | - |
| W-11 capsules:algae 50:50 | 4.9 | 5.0 | +0.1 |
| W-11 capsules:algae 75:25 | 5.3 | 5.3 | - |
| Unfed control | 5.2 | 5.0 | -0.2 |
| Initial | 4.3 | - | - |

[a] Amaizo W-11 and VII Starch (American Maize Products Co.).

[b] Algae used was Pseudoisochrysis paradoxa (VA-12).

Table 12. Average (N = 100) initial ash free dry weight (AFDW) for juvenile Pacific oysters and after treatment with seven feeding regimes (Experiment 4).

| Treatment | AFDW (mg) |
|---|---|
| Unfed control | 0.051[a] |
| Starch (W-11)[b] | 0.095[d] |
| Capsules | 0.101[d] |
| Initial | 0.101[d] |
| Capsules:algae[c] 75:25 | 0.143[a, e] |
| Capsules:algae 25:75 | 0.146[a, e, f] |
| Capsules:algae 50:50 | 0.156[a, e, f] |
| Algae | 0.159[a, f] |

[a]Significantly different from initial weight at 95% level

[b]Amaizo W-11 Starch (American Maize Products Co.).

[c]*Pseudoisochrysis paradoxa* (VA-12).

[d, e, f]No significant difference at the 95% level.

oysters fed combinations of encapsulated algae and whole algae cells showed a significantly greater AFDW than the initial sample and there was no significant difference among them. Oysters fed only whole algae cells had an AFDW that was not significantly different from the AFDW of the two treatments given the higher supplementation with algae (i.e. 50 and 75%). The unfed control sample lost a significant amount of AFDW (Table 12). All of the treatments showed no or very little shell growth during this experiment (Table 13).

## Experiment 5

The treatments fed algae only or combined with encapsulated algae showed significantly greater AFDW than the initial sample (Table 14). The AFDW of the oysters fed encapsulated algae and Sulmet was significantly larger than that of the initial sample, while the AFDW of the oysters fed encapsulated algae alone was not significantly different from the AFDW of the initial sample. The unfed control sample lost a significant amount of AFDW as compared to the initial sample. None of the treatments showed shell growth during this experiment (Table 15).

Table 13. Average (N = 100) length for juvenile Pacific oysters at beginning and end of seven feeding regimes (Experiment 4).

| Treatment | Length (mm) | | |
|---|---|---|---|
| | Beginning | End | Difference |
| Initial | 3.6 | - | - |
| Unfed control | 3.6 | 3.5 | -0.1 |
| Algae[a] | 3.6 | 3.8 | +0.2 |
| Capsules[b]:algae 25:75 | 3.7 | 3.8 | +0.1 |
| Capsules:algae 50:50 | 3.6 | 3.9 | +0.3 |
| Capsules:algae 75:25 | 3.7 | 3.8 | +0.1 |
| Capsules | 3.6 | 3.6 | - |
| Starch (W-11)[c] | 3.7 | 3.7 | - |

[a] Pseudoisochrysis paradoxa (VA-12).

[b] Amaizo W-11 Starch (American Maize Products Co.) encapsulated algal lysate.

[c] Amaizo W-11 Starch (American Maize Products Co.).

Table 14. Average (N = 100) initial ash free dry weight (AFDW) for juvenile Pacific oysters and after treatment with eight feeding regimes (Experiment 5).

| Treatment | AFDW (mg) |
|---|---|
| Unfed control | 0.062[a] |
| Capsules[b] | 0.084[f] |
| Initial | 0.086[f] |
| Starch (W-11)[c] | 0.101[a] |
| Algae[d] | 0.114[a, g] |
| Capsules + Sulmet[e] | 0.114[a, g] |
| Capsules:algae 25:75 | 0.121[a, g] |
| Capsules:algae 50:50 | 0.125[a, g] |
| Capsules:algae 75:25 | 0.142[a] |

[a]Significantly different from initial weight at 95% level.

[b]Amaizo W-11 Starch (American Maize Products Co.) encapsulated algal lysate.

[c]Amaizo W-11 Starch (American Maize Products Co.).

[d]*Pseudoisochrysis paradoxa* (VA-12).

[e]Sodium sulfamethazine.

[f, g]No significant difference at the 95% level.

Table 15. Average (N = 100) length for juvenile Pacific oysters at beginning and end of seven feeding regimes (Experiment 5).

| Treatment | Length (mm) | | |
|---|---|---|---|
| | Beginning | End | Difference |
| Initial | 3.3 | - | - |
| Unfed control | 3.4 | 3.3 | -0.1 |
| Algae[a] | 3.4 | 3.4 | - |
| Capsules[b] | 3.4 | 3.3 | -0.1 |
| Capsules:algae 75:25 | 3.6 | 3.5 | -0.1 |
| Capsules:algae 50:50 | 3.5 | 3.5 | - |
| Capsules:algae 25:75 | 3.4 | 3.5 | +0.1 |
| Starch (W-11)[c] | 3.6 | 3.5 | -0.1 |
| Capsules + Sulmet[d] | 3.5 | 3.4 | -0.1 |

[a] *Pseudoisochrysis paradoxa* (VA-12).

[b] Amaizo W-11 Starch (American Maize Products Co.) encapsulated algal lysate.

[c] Amaizo W-11 Starch (American Maize Products Co.).

[d] Sodium sulfamethazine.

# DISCUSSION

The results of Experiment 1 indicate that ground, dried oyster meat does not promote as great an increase in AFDW of juvenile C. gigas as algae. The oyster meat may have had essential nutrients only in limited quantities. One possibility is that the unsaturated fatty acids in the oyster meat (Watanabe and Ackman, 1972) became oxidized as a result of the drum drying and grinding. The hydroperoxides and other products produced by the degradation of the unsaturated fatty acids possibly did not have the same nutritional value to the oyster as the original lipids. It has been found that severe oxidation of lipids yields products that produce severe physiological damage when fed to test animals (Dugan, 1976).

The average length measurement observed at the beginning of the experiment for the agar fed treatment was 6.9 mm and at the end was 6.5 mm. The apparent reduction in shell length in this group may have been due to shell breakage as these shells seemed to flake more easily when handled during measurement than those in the other treatments.

The agar fed oysters lost a significant amount of AFDW while the unfed oysters did not. The agar may have been indigestible and most probably was nutritionally incomplete for oysters. Possibly food reserved are depleted by the filtering and handling of particles

that are not digestible or are nutritionally incomplete. This may explain the lack of shell or meat growth that occurs when larval and juvenile oysters are fed certain species of phytoplankton such as Prymnesium parvum (DeMort, 1970).

The AFDW and shell length of the unfed oysters increased during the course of Experiment 1, but these gains in weight and length were not significantly greater than the values for the initial sample. The small amount of growth shown by the unfed oysters, although not significant, may have been due to the assimilation of dissolved organics or bacteria as it was not possible to totally eliminate these factors given the design of the experiment.

The results of Experiment 2 may be confounded by the effects of a water toxicity problem that often has occurred at this time of year at the Oregon State University experimental oyster hatchery (R. E. Malouf, personal communication), as none of the oysters gained a significant amount of AFDW. The oysters fed lysed algae lost a significant amount of AFDW. In all of the other experiments, the oysters fed algae gained a significant amount of AFDW.

The dried algae fed to oysters in Experiment 3 did not support shell or meat growth. This result agrees with the results of experiments by Hidu and Ukeles (1962) during which it was determined that an alga such as Isochrysis galbana, which normally supports good larval shell growth, did not support good growth when fed to larvae in

a freeze-dried form. As Isochrysis galbana contains a high percentage of highly unsaturated fatty acids (DeMort, 1970), the possibility exists that these fatty acids became oxidized during the drying process and were no longer available to the oyster in an essential form.

The results of Experiment 3 indicate that encapsulated lysed algae promotes the same amount of oyster meat growth as whole algae cells. However, the results of the fourth and fifth experiments indicate that the encapsulated lysed algae is not nutritionally adequate for oysters under the conditions of these experiments. It is difficult to compare the results of Experiment 3 to those of Experiments 4 and 5 because of the different conditions under which they were conducted.

Experiments 4 and 5 were conducted in a constant temperature room, while Experiment 3 was conducted in an aquarium room where the water temperature varied from 12.2-20.5 C. One possible reason for the differing results of Experiment 3 and Experiments 4 and 5 is that the temperature range of 15-17 C, although close to the optimal temperature (15 C) for the consumption of algae by juvenile C. gigas (Malouf, 1977), may not be an optimal range for the consumption of the starch used to encapsulate the algal lysate.

In Experiments 4 and 5, oysters were fed a different batch of capsules from that fed to oysters in Experiment 3. The oysters in Experiments 4 and 5 were fed algae for two weeks prior to the start of the experiments, while those in Experiment 3 were not. I assumed

that by feeding the oysters for two weeks prior to the start of the experiment that the oysters would become conditioned to filtering algae and that this would allow time for the appropriate digestive enzymes to be induced. Possibly the enzymes induced by feeding algae are not suitable for digesting the starch encapsulated algae also.

Experiments 4 and 5, which were conducted under the same conditions, gave similar but not identical results. In both experiments, the unfed control samples lost a significant amount of AFDW, the AFDW of the oysters fed the encapsulated diet was not significantly different from that of the initial sample, and the oysters fed either algae alone or algae supplemented with encapsulated algae showed significantly greater AFDW from the initial sample.

In Experiment 4, when oysters were fed the starch used to encapsulate the algae, the resulting average AFDW was not significantly different from that of the initial sample. In Experiment 5, the AFDW of the starch fed sample was significantly greater than that of the initial sample. The increase in AFDW shown by the starch fed oysters in Experiment 5 may have been due to the starch being supplemented by dissolved organics or bacteria that may have been present in greater quantities during this experiment that during Experiment 4. As mentioned previously, it was not possible to totally eliminate dissolved organics or bacteria from the culturing water.

The second batch of the encapsulated diet used in Experiments 4 and 5 failed to significantly increase the AFDW of oysters, while the algae fed oysters consistently showed an increase in AFDW. When Sulmet was added to the culture water in Experiment 5, the oysters fed the capsules showed AFDW not significantly different from the AFDW of the oysters fed algae. Sulmet may have contributed some nutritional factors (J. E. Lannan, personal communication).

# SUMMARY

The encapsulated diets appear to be a suitable vehicle for delivery of synthetic diets to juvenile oysters. While the encapsulated diets did produce an increase in AFDW under certain experimental conditions, they failed to consistently produce increases in AFDW of juvenile oysters under all of the experimental conditions tested. When the animals fed the encapsulated diets did not show a significant increase in AFDW, they did not lose a significant amount of AFDW, as was observed for the unfed controls. It is therefore suggested that these diets could be used as a maintenance ration in hatcheris if phytoplankton cultures fail to grow or become badly contaminated by bacteria. More research needs to be done before these diets can replace algae for culturing oysters.

Although the process of microencapsulation appears to be a promising solution to the problem of feeding juvenile oysters in the hatchery, there are many factors that need to be considered in the formulation of the capsules. One problem is that the capsules need to be the proper size. The capsules should have a coating that is impermeable to seawater and yet is digestible to the oyster. The capsules should be either neutrally buoyant or else a means of suspending the capsules needs to be supplied. Finally, the two most important factors to consider are that the capsules should produce optimal growth and be less expensive to feed than algae. Until these criteria are met, microencapsulated foods will not be able to replace algae for culturing oysters.

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