ABSTRACT

Theresa Bosse Hurd. EFFECTS OF FATTY ACID SUPPLEMENTS TO A MINIMAL DIET ON GROWTH OF THE HARD CLAM, Mercenaria mercenaria (L.). (Under the direction of Margie Lee Gallagher) Department of Biology, November 1985.

Juvenile clams (Mercenaria mercenaria (L.)) collected near Cedar Island, Virginia were placed in experimental containers with artificial seawater were aerated then subjected to three different diets. Diet 1 (minimal diet) consisted of brewer's yeast and freeze-dried algae (Isochrysis galbana) in a 1/1 ratio by wt. Diet 2 consisted of the minimal diet with a 5% corn oil supplement. Diet 3 consisted of the minimal with a 5% cod liver oil supplementation. The diets were analyzed for ash, protein, lipid and fatty acid content. After 28 d clams were analyzed for change in whole wt, length, ash, protein and lipid. The minimal diet maintained slow but steady growth for three wk. Clams fed diet 2 had a greater increase in shell length (9.30 ± 0.10 mm, $p < 0.05$) than clams fed either diet 1 (3.22 ± 1.30 mm) or diet 3 (8.45 ± 0.70 mm). Clams fed diet 2 had a greater increase in whole wt than clams fed diets 1 or 3. Although not significant in most replications, clams fed diet 3, the minimal diet and 5% cod liver oil supplementation, had greater shell lengths and whole wt than clams fed diet 1. Clams receiving diet 2 and

diet 3 contained significantly ($p < 0.05$) greater % crude fat than the clams receiving the minimal diet. There were no significant differences in protein and ash content of clams fed the three diets. However, ash contents for all diets were significantly greater than in the initial clam sample, indicating shell deposition. These results may be due to differences in fatty acid content of corn oil and cod liver oil since diet 2 with the corn oil supplementation had more than ten times as much 18:2 w6 fatty acid than any other diet. Although cod liver oil contained three times as much 18:3/20:1 w3 fatty acids than diet 2, the minimal diet appeared to supply a sufficient amount of w3 fatty acids.

EFFECTS OF FATTY ACID SUPPLEMENTS TO A MINIMAL DIET ON GROWTH OF THE HARD CLAM, *Mercenaria mercenaria* (L.)

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INTRODUCTION

Interest in mariculture of the hard clam or quahog Mercenaria mercenaria (L.) has increased along the east coast of the United States as prices for this commercially important species have increased and harvest of natural clam beds has decreased (Foster, 1981). Juvenile clams must be purchased from commercial hatcheries, to increase the density of clam populations in estuarine areas. Consequently, most of the work on nutritional needs of clams concerned the larval stages. Work has been done to identify nutritional requirements of fish and decapod crustaceans but similar nutritional studies of bivalve mollusks are rare (Langdon, 1981a and b).

Food is obtained from natural processes when estuarine waters are used in culturing bivalves (Epifanio et al., 1975a). Once nutritional requirements of bivalve mollusks are known, an artificial diet can be developed. Such a diet mixture would facilitate laboratory and/or commercial rearing of bivalves and initiation of genetic studies (Castell and Trider, 1974). Thus identification of nutritional needs could lead to production of healthier, disease resistant clams with improved taste and appearance. Several species of algae have been identified as good food sources for larval and juvenile M. mercenaria (Loosanoff and Davis, 1963; Walne, 1970; Epifanio, 1979a). There is no

known artificial diet to support growth of M. mercenaria comparable to growth obtained with live algal diets (Langdon, 1981a).

Purpose

Studies with juvenile M. mercenaria and Crassostrea virginica indicate that fatty acids are important in shellfish nutrition (Dunathan et al., 1969; Trider and Castell, 1980; Castell and Trider, 1974). This study compares growth of juvenile M. mercenaria fed diets of 50% freeze-dried unicellular algae (Isochrysis galbana) and 50% brewer's yeast supplemented with different combinations of fatty acids as corn oil and cod liver oil. Gross composition of the dietary food in terms of protein, fat, ash, and fatty acid content is related to growth of M. mercenaria. Shell length, whole wet wt, protein, fat, and ash content were used as indices of clam growth.

LITERATURE REVIEW

Phytoplankton Diets

For 42 d Epifanio (1979a) fed juvenile *M. mercenaria* diets of various combinations of the algae *Cartesis chui*, *Isochrysis galbana*, *Platymonas suecica* and *Thalassiosira pseudonana*. Growth was measured as increase in shell length and dry wt of soft tissue. *M. mercenaria* had good growth when fed a combination diet of *I. pseudonana* and *I. galbana*. Either species (*I. pseudonana* and *I. galbana*) as a sole dietary component gave intermediate growth which was less than their combination. Little growth occurred with *C. chui* and *P. suecica* as sole dietary components or in combination with each other. This could be due to the absence of a micronutrients in *C. chui* or *P. suecica* which are necessary for growth.

Walne (1970) surveyed 19 genera of algae as possible food for juvenile bivalves and found that *Tetraselmis suecica*, *Pyramimanas grossii* and *Skeletonema costatum* were as good or better as food than *I. galbana*. Maximum growth was with concentrations of *I. galbana* between 10 to 25 cells per microliter and growth was not enhanced at 50 cells per microliter. Growth of clams was unfavorable when they were fed at a concentration of 75 cells per microliter. Studies previous to that of Walne (1970) were with diets for bivalve larvae and it had been generally assumed that foods that

were good for larvae would also be adequate for juveniles.

Walne (1970) found that freeze-dried I. galbana fed to Ostrea edulis was not an adequate diet when compared to a diet of live I. galbana. When the dried cells were suspended in artificial seawater by shaking there were some clumps of 2-12 cells.

Mann and Ryther (1977) found little potential for cultivating M. mercenaria in effluent from a waste recycling aquaculture system. The slow growth was attributed to poor food quality of the diatom Phaeadoctylum tricornutum which was the predominant species.

Phytoplankton and Yeast Diets

Epifanio (1979b) fed M. mercenaria diets of varying proportions of the yeast Candida utilis and the diatom T. pseudonana. When as much as 50% of the diet consisted of yeast, growth over 28 d was comparable to that of controls which were fed 100% algae.

Addition of yeast to algal rations supported slow but sustained, short-term growth in juvenile M. mercenaria (Urban, 1982). The yeast products, Pur-Culture-Py and Torula Dried Yeast, were considered the best yeast supplements for clams. However, Urban (1982) did not confirm the findings of Epifanio (1979b) that diets of up to 50/50 yeast and algae support clam growth as well as a full algal diet. Urban (1982) attributes these differences to

different culture conditions and to a growth increment of 100% for dry meat wt observed by Epifanio (1979b) compared to the 750% increase in dry wt in his study.

Artificial Supplements

More work has been done in development of an artificial diet for oysters than for clams. Haven (1965), in a supplemental feeding experiment with C. virginica, concluded that quantity of carbohydrate in planktonic algal cells influences tissue wt of adult oysters. The addition of about 5 mg/l cornstarch or wheat flour to York River, Virginia water flowing at optimum rates significantly increased tissue wt of oysters during the fall. Consequently starch may be used to increase meat in oysters prior to harvesting.

Castell and Trider (1974) fed C. virginica artificial diets of known nutrient composition. Using increases in meat wt, and glycogen content as growth parameters, they found that the higher levels of dietary carbohydrate resulted in greater glycogen production. However, Epifanio (1979b) found that the mean growth of C. virginica could not be related to the gross chemical composition of diets of varying amounts of yeast and algae. Castell and Trider (1974) found that diets containing cod liver oil resulted in greater glycogen content production for C. virginica than the diets containing corn oil. Also diets containing cod

liver oil gave a significant live wt gain at 10 wk over the oysters fed diets containing corn oil.

Dunathan et al. (1969) analyzed the glycogen accumulation in adult oysters (C. virginica) fed an artificial diet of cornmeal, brown rice, barley, hominy, cornstarch, millet, torula yeast, crab meat, whole wheat, cellulose, glucose, aggregated glucose, the alga Gracilaria sjoestedtii, and combinations of cornmeal/crab meat, and cornmeal/brown rice. The best results were with cornmeal and rice when the total carbohydrate content of the diet was between 67.0% and 75.6% and the maximal concentration of food was 10 mg per l. Both corn meal and brown rice have similar carbohydrate contents (75.4 and 75.6%); the rice-fed oysters had a higher glycogen content. Lipids are important functional components in cells and membranes and the fatty acid pattern of rice lipid may be a factor contributing to the superior food value of brown rice. Cod liver oil has a high content of fatty acids of the linolenic series whereas corn oil is low in linolenic oil (Castell and Trider, 1974).

Trider and Castell (1980) found diets that contained cod liver oil produced significantly greater live wt in C. virginica than did corn oil or hydrogenated coconut oil. Diets that contained a high level of a w3 type fatty acids (cod liver oil) were of greater significance than diets with mainly w6 type fatty acids (corn oil). A mixture of a w3 type fatty acid and fatty acids which contained w6 type

fatty acid did not produce a greater difference in % of dry wt than cod liver oil alone. Bivalves in general tend to utilize dietary fatty acids in respiration, accumulation as triglycerides, transfer to phospholipids, or modification as phospholipids (Ackman, 1982).

MATERIALS AND METHODS

Algal Cultures

An initial algal culture (*Isochrysis galbana*) was purchased from the University of Texas Algal Culture Collection. The algae was maintained in 3.8 l glass containers in seawater 30 g/l salinity collected from Oregon Inlet, Pamlico Sound, North Carolina. The seawater was autoclaved, Alga Gro and a pinch of $CaCO_3$ were added to the supernant. Light was supplied by two Vita-lite fluorescent four foot lamps. A stock culture was maintained under sterile conditions and an intermediate culture was used to inoculate the culture containers. Air was bubbled continuously through an inlet valve at the top of the container while a second valve served as an outlet for air. The cultures were inspected periodically microscopically for purity and vitality of the culture. Harvest was at the stationary phase by centrifugation to concentrate the algae which were then frozen and later freeze-dried.

Since required temperature of 18°C could not be maintained, the initial algal culture of *I. galbana* was replaced with Tahitian *I. galbana* which tolerated the higher temperatures between 20°C to 25°C. Available centrifuges were not adequate to concentrate enough algae and when a source of *I. galbana* was obtained culturing was abandoned.

Source and Handling of Mercenaria mercenaria

Juvenile clams, M. mercenaria were obtained from the Virginia Institute of Marine Science in Wachapreague, Virginia. The clams, from the wild stock collected near Cedar Island, were treated with Combiotic about two wk prior to the experiment. The initial mean whole wet wt were 0.44 ± 0.15 (SEM) g and the initial mean lengths were 1.06 ± 0.02 (SEM) cm.

Two hundred clams were randomly chosen. Fifty were used for measurement of initial growth parameters. One-hundred-fifty clams were placed in experimental containers of artificial seawater and air with ten clams per container to avoid crowding. Five groups of ten clams were fed one of the three different diets. The clams were marked with fingernail polish to identify individuals.

Dietary Components

Isochrysis galbana and Yeast. The algae were obtained from the Horn Point Laboratory in Cambridge, Maryland. The algal sample was frozen and later freeze dried. To freeze dry, the frozen algal samples were placed in a vacuum chamber of a Labcono freeze drier at -50°C and a high vacuum was applied.

Brewer's yeast and I. galbana were used for 50/50 diet of yeast and algae. Diet 1 served as the control and a minimal diet consisted of 50/50 yeast and algal ration with

no fatty acid supplement.

Fatty Acid Supplements. Fatty acids were added in the form of corn oil and cod liver oil. Corn oil was used as a supplement to the yeast/algal ration in Diet 2. Cod liver oil was used to supplement the yeast/algal ration in Diet 3. In Diets 2 and 3 the oil was 5% of the total dry wt of the yeast/algal ration. The oils were added to the dried algae which completely absorbed them. Microscopic observations of the hydrated yeast/algae/oil ration gave no evidence that any oil had separated from the algae. Also, oil was not seen in preliminary observations of the yeast/algae/oil ration in the aquaria.

General

Rations. The food ration was calculated using the equation:

$$Q_R = 0.01\ W^{-0.33}$$

where Q_R is g dry wt of ration per g whole wet wt of clam and W is g whole wet wt of clam. Epifanio and Ewart (1977) used this equation to estimate an optimum ratio for C. virginica at 20°C. Individual clams were weighed weekly to adjust food ration to increasing wt gain. The food ration was blended weekly in 350 ml of artificial seawater for 5 min at high speed to break up clumps and obtain a suspension of cells. The food ration was stored at 5°C.

Seawater, Environmental Factors, and Aquaria.

Artificial seawater was made from "Instant Ocean" which was dissolved in tap water and bubbled with air for 24 hr to remove chlorine. A salinity of 29 g/l was maintained.

The aquaria consisted of five 1 plastic or glass containers. Ten clams were placed on the bottom of each aquaria in about 3.5 l of artificial seawater.

Air was bubbled through the aquaria constantly to keep the oxygen level near saturation. A safe level for total ammonium nitrogen is considered to be 10 mg/l for invertebrate culture systems (Epifanio et al., 1975b; Wheaton, 1977). The water was changed every three d so as not to exceed tolerance limits. The aquaria received eight hr of light from Vita-lite fluorescent lamps and 16 hr of dark. The water temperature ranged from 20°C to 24°C.

Procedure

Feeding Method. Clams were blotted dry, weighed to the nearest hundreth of a g, marked, measured with vernier calipers and randomly placed in groups of five with ten clams per group for each diet. The daily food ration was stirred on a magnetic stirrer and measured in a graduated cylinder. Five ml per clam was added to each group.

Analyses. After 28 d wt and length were again determined. The whole clams were then placed in test tubes according to groups, frozen, and freeze-dried. The

freeze-dried clams were ground in a Wiley intermediate mill with a 20-mesh sieve size. Crude lipid extraction was by the Soxhlet procedure with chloroform and methanol (2:1) (Gallagher et al., 1984). Protein determination was by the Biuret method (Gornall et al., 1949). Bovine Serum Albumin was used to determine a standard curve (Gallagher et al., 1984). The fat extracted sample was ashed at 600°C to a constant wt.

Fatty acid analysis was by gas chromatography of methyl ester derivatives from the extracted lipid (Medwadowski et al., 1967). Chromatographic grade reagents and gases were used in the fatty acid analysis. Analyses were with a Varian Model 3700 gas chromatograph with a 10% SP-2330 100/200 Suplecoport column and flame ionization detector with N_2 as the carrier gas. Peak area identification was according to Gallagher et al. (1984). Retention times were compared to standard fatty acid methyl ester mixtures obtained from Supelco, USA. The standards were run separately and were also incorporated in esterified corn oil and cod liver oil. Fatty acids were then identified by the comparison with two different standards which contained the known peak of interest.

Water Clarity and Feeding. Observations were made on containers where food was added without clams to determine the settling time. Observations on siphoning time were made

in containers that contained clams with and without food.

Statistical Analyses. Each experiment was terminated at the end of 28 d because there was a significant difference between the initial whole wt and the whole wt of one of the control groups after 21 d using a Student's t-test (Zar, 1974). Differences among mean individual whole wet wt and lengths of five groups on each diet were analyzed by one-way analysis of variance test for statistical significance between replications. Differences in clam growth between diets using the growth parameters wt and length were analyzed for statistical significance by one-way analysis of variance using the Duncan's Multiple Range test (Walpole and Myers, 1978). Differences in clam growth between diets using ash, protein, and fat as growth parameters were analyzed for statistical significance by one-way analysis of variance using the Duncan's Multiple Range test.

RESULTS

Analyses of Isochrysis galbana and Brewer's Yeast

Nutrient analyses of the two single cell cultures (diet 1) used in this study are shown in Table 1. I. galbana contained 68.36 ± 2.79 (SEM) % crude fat compared to the 6.46 ± 0.11 % crude fat for brewer's yeast. I. galbana contained 20.14 ± 2.51 % protein. Brewer's yeast contained 29.82 ± 0.65 % protein. I. galbana was 14.90 ± 2.51 % ash where brewer's yeast was 8.05 ± 0.04 % ash.

After three wk there was a significant ($p < 0.05$) wt gain by control clams. Therefore, the diet (1/1) of freeze dried I. galbana and brewer's yeast maintained slow but steady growth in juvenile M. mercenaria for three wk.

Growth of Clams on Artificial Diets

Fat. The clams fed diets 2 and diet 3 had significantly ($p < 0.05$) greater % crude fat than the clams receiving diet 1 (control) (Table 2). The clams fed diets 2 and 3 were not significantly different. All the clams fed the artificial diets had lower fat levels than the initial clam sample. Only those clams fed diet 2 supplemented with corn oil had fat levels that were not significantly different from the initial sample.

Ash. The % ash content in the initial clam sample was significantly lower than the % ash content of clams fed test

TABLE 1

Mean % (SEM) composition of selected dietary components of *Isochrysis galbana*[1] and brewer's yeast[2] on a dry wt basis.

Dietary Component	*Isochrysis galbana*	Brewer's Yeast
Fat (Chloroform/methanol extraction)[3]	68.36 ± 2.79	6.46 ± 1.78
Protein (Biuret Method)[4]	20.14 ± 2.51	29.82 ± 2.10
Ash (600°C)	14.90 ± 2.51	8.05 ± 0.46

[1] *Isochrysis galbana* was obtained from the Horn Point Laboratory in Cambridge, Maryland and freeze-dried at -50°C under a vacuum.

[2] Brewer's Yeast was obtained from Lewis Laboratories, 49 Richmondville Avenue, Westport CT 06880.

[3] Crude lipid extraction was determined by the Soxhlet procedure with chloroform and methanol (2:1) (Gallagher et al., 1984).

[4] Protein determination was with the Biuret Method (Gornall et al., 1949). BSA was used to determine a standard curve (Gallagher et al., 1984).

TABLE 2

Mean % (SEM) fat, protein, ash and moisture content of clams initially receiving *Isochrysis galbana* and brewer's yeast (50/50) alone (diet 1) or supplemented with either 5% corn oil (diet 2) or 5% cod liver oil (diet 3)

	Fat	Protein	Ash	Moisture
Initial	3.31 ± 0.06^{c} [1]	7.56 ± 0.37^{a}	90.69 ± 1.02^{a}	67.09 ± 4.42
Diet 1	2.19 ± 0.19^{a}	7.31 ± 0.28^{a}	92.95 ± 0.20^{b}	65.31 ± 1.63
Diet 2	3.05 ± 0.13^{bc}	6.80 ± 0.45^{a}	93.95 ± 0.17^{b}	65.29 ± 2.90
Diet 3	2.66 ± 0.10^{b}	7.29 ± 0.19^{a}	93.66 ± 0.47^{b}	65.17 ± 1.72

[1] Means with the same superscripts are not significantly different from one another at $p < 0.05$.

diets ($p < 0.05$) (Table 2). The % ash content of clams fed diets 1, 2, or 3 were not significantly different from each other.

Protein. There was no significant difference in % protein among clams in the initial sample or in those fed the test diets (Table 2).

Length and Weight. An analysis of variance was used to check for significant differences between the five groups used in this study. Group 2 was significantly different ($p < 0.05$) from all other groups of clams fed diet 1. Since each group contained ten identifiable individuals, it was possible to complete the statistical analyses for change in wt and length for group 2 in all diets separately from groups 1, 3, 4, and 5. Therefore, for the subsequent references to analyses of groups 1, 3, 4 and 5 will be referred to as experiment 1 and the analyses of group 2 will be referred to as experiment 2.

For experiment 1, the change in wt for clams receiving diet 2 was significantly ($p < 0.05$) greater than for diet 1 (Table 3). Although clams receiving diet 3 had higher wt gains than those receiving the control diet, clams receiving diet 3 were not significantly different from diets 1 and 2.

Increase in shell length was significantly greater for clams receiving diets 2 and 3 than for clams on diet 1 (Table 3).

TABLE 3

Mean change in weight and length of clams (SEM) fed *Isochrysis galbana* and brewer's yeast (50/50) alone (diet 1) or supplemented with either 5% corn Oil (diet 2) or 5% cod liver oil (diet 3) in experiment 1

	n	Mean Initial Weight (g)	Mean Increase in Weight (g)	Mean Initial Length (cm)	Mean Increase in Length (cm)
Diet 1	32	0.47	0.03 ± 0.00^{a} [1]	1.08	0.03 ± 0.00^{a}
Diet 2	30	0.46	0.05 ± 0.01^{b}	1.10	0.09 ± 0.00^{b}
Diet 3	29	0.39	0.04 ± 0.01^{ab}	1.03	0.08 ± 0.01^{b}

[1] Means with the same superscripts are not significantly different from one another at $p < 0.05$.

In a one-way analysis of variance for experiment 2 there was no significant ($p > 0.05$) difference in the wt gain for clams receiving diets 1, 2 or 3. However, clams receiving diets 2 and 3 had a greater increase in length than clams receiving diet 1 (Table 4).

Fatty Acid Identification. The minimal diet of dried I. galbana and brewer's yeast provided a combination of 11 different fatty acids (Table 5). The brewer's yeast did not provide any fatty acid not provided by I. galbana. It did supply three times as much 16:1 and 18:1 than was provided by the dried I. galbana. I. galbana provided four fatty acids that were not found in brewer's yeast: 14:0, 14:1, 20:2, and 22:6 (tentative identification). The minimal diet with the cod liver oil supplement provided a combination of 14 different fatty acids. Cod liver oil supplied three fatty acids that were not provided by the minimal diet: 20:5, 22:0, and 22:1. The corn oil supplement did not provide any different fatty acids than were already provided by the minimal diet. However, the corn oil had more than ten times as much 18:2 than any other source.

Water Clarity and Feeding. Within 24 hr after the addition of food to the aquaria with clams, the water was completely cleared. Particles which were assumed to be feces and pseudofeces collected on the sides as well as the bottom of the aquaria. Four to six hr following the

TABLE 4

Mean change in weight and length (SEM) of clams fed *Isochrysis galbana* and brewer's yeast (50/50) alone (diet 1) or supplemented with either 5% corn oil (diet 2) or 5% cod liver oil (diet 3) in experiment 2

	n	Mean Initial Weight (g)	Mean Increase in Weight (g)	Mean Initial Length (cm)	Mean Increase in Length (cm)
Diet 1	9	0.50	0.08^{a}[1] ± 0.01	1.12	0.05^{a} ± 0.01
Diet 2	10	0.48	0.07^{a} ± 0.02	1.01	0.19^{b} ± 0.04
Diet 3	8	0.39	0.05^{a} ± 0.08	1.04	0.08^{a} ± 0.02

[1] Means with the same superscripts are not significantly different from one another at $p < 0.05$.

TABLE 5

Fatty acid composition expressed as percentage of total fatty acids (SEM) of the dietary components: *Isochrysis galbana*[1], brewer's yeast[2], corn oil[3] and cod liver oil[3].

Fatty Acid[4]	*Isochrysis galbana*	Brewer's Yeast	Corn Oil	Cod Liver Oil
14:0	19.37 ± 1.18	trace	———	7.20 ± 0.54
14:1	trace	———	———	———
16:0	17.93 ± 1.52	18.6 ± 0.65	10.68 ± 2.84	17.02 ± 0.90
16:1	8.39 ± 0.89	31.38 ± 3.12	———	15.64 ± 0.99
18:0	2.83 ± 0.03	7.40 ± 0.41	1.51 ± 0.2	2.84 ± 0.62
18:1	8.97 ± 0.39	39.27 ± 1.59	27.2 ± 0.2	32.56 ± 0.99
18:2	5.33 ± 0.42	3.06 ± 0.48	58.2 ± 1.0	5.68 ± 0.19
18:3/20:1	2.54 ± 0.21	trace	———	11.96 ± 1.20
20:2	8.99 ± 0.68	———	———	———
20:5	———	———	———	2.03 ± 0.38
22:0	———	———	———	0.09 ± 0.12
22:1	———	———	———	5.10 ± 1.13
Tentative 22.6	25.66 ± 0.49	———	———	———

[1] *Isochrysis galbana* was obtained from the Horn Point Laboratory in Cambridge, Maryland and freeze-dried at -50°C under a vacuum.

[2] Brewer's yeast was obtained from Lewis Laboratories, 49 Richmond Avenue, Westport, CT 06880.

[3] Corn oil and cod liver oil were obtained from National Biochemicals, P.O. Box 28050, Cleveland, Ohio 44128.

[4] Fatty Acid analysis was determined by gas chromatography of methyl ester derivatives from the extracted lipid (Medadowski et al., 1967). Notation for fatty acids is: 14:1 indicates 14 carbons, and 1 double bond.

addition of food to aquaria without clams the food started to settle; however, after 48 hours the food was not completely settled and the water was not clear. In aquaria with food but no clams, there was no accumulation of particles on the sides of the aquaria. The particles that accumulated on the bottom and sides of the aquaria with clams were much larger than the food particles.

DISCUSSION

Dried Yeast/Algal Diets

Freeze dried *I. galbana* and brewer's yeast supported slow, steady growth of *M. mercenaria* for up to three wk as indicated by weekly weighing. It has been reported that dried algae were not as good a food as live algae for the oyster species, *Ostrea edulis* (Walne, 1970). The drying process may reduce digestibility of the algae as compared to live algae. Urban (1982) found that the yeast product Pur-Culture-Py, in combination with live algae, supported growth for three wk, and Epifanio (1979b) found that diets containing up to 50% of the yeast *Candida utilis* and 50% live algae supported growth comparable to 100% algae. Urban (1982) attributed the yeast with supplying macronutrients as protein and the algae with supplying essential micronutrients that were not supplied by the yeast. The analyses of yeast and algae in this study do not support this suggestion. The 20.14% protein supplied by the freeze-dried *I. galbana* is comparable to the 29.82% supplied by the brewer's yeast (Table 1). The freeze-dried algae, however, supplied 68.36% fat compared to only 6.46% fat supplied by the brewer's yeast (Table 1). The *I. galbana* may, however, have supplied micronutrients. This is the first report of sustained growth of *M. mercenaria* on a mixed dried yeast/algae diet. The slow growth supported in this

study allowed the effects of dietary supplements to become evident.

Fatty Acid Supplementation

The diets supplemented with corn oil and cod liver oil supported better growth than the diet of freeze dried algae and brewer's yeast alone. The corn oil supplementation was generally of a greater nutritional value than the cod liver oil supplementation. This result does not agree with the work of Trider and Castell (1974 and 1980) who reported that cod liver oil supported live wt growth in Crassostrea virginica better than corn oil in diets where corn oil or cod liver oil supplied the major portion of lipid in the diet. One reason for this difference may be that in this study the major portion of lipid was supplied by I. galbana which contained 68.36% fat. I. galbana has been reported to be high in fatty acids of the linolenic type (Demort et al., 1972). Cod liver oil is also considerably higher in fatty acids of the linolenic series than corn oil (Castell and Trider, 1974). Therefore, cod liver oil most likely increased essential fatty acids already supplied by I. galbana where corn oil may have supplied fatty acids extremely limited or lacking in both cod liver oil and I. galbana. In addition, while Trider and Castell (1980) found that cod liver oil alone produced significantly greater wet wt than corn oil or a mixture of cod liver oil and corn oil,

the combination of cod liver oil and corn oil produced a greater % dry wt in oysters fed both of these lipids.

Dunathan et al. (1969) found the best accumulation of glycogen in adult oysters C. virginica when fed a combination of cornmeal and rice with a total carbohydrate content between 67.0 and 75.6%. Though both grains have a similar carbohydrate content the rice-fed oysters had the highest glycogen content. Castell and Trider (1974) attributed the better growth from rice to the fatty acid pattern which must contain a factor that results in its superior food value. The results of this study indicate that M. mercenaria exhibit better growth when fed a combination of fatty acids found in both cod liver and corn oil.

Diet 2 with the corn oil supplement provided more than three times as much 18:2 (linoleic acid) than either diet 1 or diet 3 (Table 6). Diet 3 with the cod liver oil supplement provided more than two times as much 18:3 (linolenic acid)/20:1 than either diet 1 or diet 2 (Table 6). Takeuchi et al. (1980) found the best wt gain in the eel Anguilla japonica was obtained by feeding a diet of 0.5% of both 18:2 and 18:3. The 18:2 fatty acid is converted into 20:4 and 22:5 and 18:3 fatty acid is converted into 22:6 (Hoar et al., 1979 and Takeuchi et al., 1980). This conversion was found to be inhibited if the dietary fatty acids 18:2 and 18:3 were deficient (Takeuchi et al., 1980).

TABLE 6

Milligrams of fatty acid for the fatty acids: 16:0, 18:0, 18:1, 18:2, and 18:3/20:1 per g of dried *Isochrysis galbana* and brewer's yeast (50/50) alone (diet 1) or supplemented with either 5% corn oil (diet 2) or 5% cod liver oil (diet 3).

Fatty Acid	Diet 1	Diet 2	Diet 3
16:0	32.70	38.20	41.20
18:0	6.47	7.22	7.97
18:1	25.24	38.74	41.74
18:2	8.91	37.91	11.91
18:3/20:1	3.88	3.88	9.88

Linolenic acid was found to be more important than linoleic acid (Hoar et al., 1979 and Takeuchi et al., 1980).

The fatty acids 20:4 and 22:5 were not supplied by any dietary source but the fatty acid 22:6 was supplied by the dried algae. In diet 2 there was a large increase in 18:2 and 22:6 was supplied by the dried I. galbana. This may explain why the corn oil supplemented clams grew better than those did with the cod liver oil supplement. Klingensmith (1982), in an analysis of fatty acids isolated from the whole clam and specific tissues, found 22:6 to be a principal fatty acid comprising about 10% of relative percentage wt composition.

The growth response in this study appeared to be due to an increase in shell deposition. The increase in shell deposition was indicated by increases in shell length and ash content in all clams (Tables 2, 3 and 4). Trider and Castell (1980) in a feeding experiment with C. virginica, also found the initial growth response to be shell formation. Generally, increase in shell length and an increase in wt gain is associated with a temperature change from 8°C to 20°C in the spring of the first year; at 10°C or below the growth of M. mercenaria was found to be negligible (Pratt and Campbell, 1956). The effect of temperature on increase in shell length and shell wt is age dependent. During the second year of growth there is a greater increase in shell wt and length compared to the third yr of growth

when the water temperature increases to 10°C. The increase in shell wt is greater than the increase in shell length during both the second and third yr of growth. This difference in shell length and shell wt is the result of two different processes. The increase in shell wt is primarily a function of ions moving across the mantle whereas the increase in shell length is a function of the extent of mantle growth (Wilbur and Yonge, 1964).

In the present study, there was an increase in wt in all clams. There was no significant increase in protein composition of experimental clams compared to the initial measurements (Table 2). There was a significant increase ($p < 0.05$) in ash content in all the experimental clams compared to the initial measurements (Table 2). All of these results are consistent with the physiological conditions described above.

In all cases clams fed diets supplemented with lipid had a significantly ($p < 0.05$) greater crude fat content (Table 2) and a greater increase in shell length than clams fed a diet lacking in lipid supplement (Tables 3 and 4). These differences may be attributed to the nutritional effect of lipid on mantle development and subsequently shell deposition, since no tissue in the clam appears to be a storage depot for fat (Klingensmith and Stillway, 1982). The greatest concentration of total lipids have been found to be concentrated in tissues where there is relationship

between tissue function and an interface between the internal and external environment. The highest concentration of total lipids have been found in the mantle and gill where both contained approximately 26% lipid on a dry wt basis (Klingensmith and Stillway, 1982).

SUMMARY

1. Freeze drying algae eliminated the need to maintain live algal cultures as the material could be stored for a long time. Concentration of a large volume of material was required to harvest a sufficient quantity of algae.
2. Freeze dried *I. galbana* and brewer's yeast provided a minimal diet in which juvenile *M. mercenaria* maintained a slow steady growth for up to three wk and was used to study to see the effects of lipid supplements.
3. *M. mercenaria* fed diets supplemented with either corn oil or cod liver oil grew significantly better (increased wt and shell length) than clams fed diets without lipid supplements.
4. There was a greater though not always significant increase in shell length and wt gain in all juvenile clams with the corn oil supplement than with the cod liver oil supplement.
5. Cod liver oil is higher in fatty acids of the linolenic series where corn oil is higher in fatty acids of the linoleic series. This study indicates that juvenile *M. mercenaria* may grow better with a combination of fatty acids found in both cod liver oil and corn oil.
6. The initial growth response appeared to be an increase in shell deposition. The greater shell formation in clams fed diets supplemented with lipid has been

attributed to the greater nutritional value of lipid on mantle development.

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