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Effects of spray-dried algae, *Schizochytrium* sp. and *Haematococcus pluvialis*, on growth and survival of Manila clam, *Tapes philippinarum*, spat were determined. Results of the present study showed that these two different spray-dried diets supported clam growth when added as supplements to rations of a mixed diet of the live algae *Isochrysis galbana* and *Chaetoceros calcitrans*. Increase in clam wet weight with 1/4 ration of live algae and a supplement of *Haematococcus pluvialis* or *Schizochytrium* sp. were similar to those of clams fed on a full live algal ration alone. This result shows that 75% substitution of live algae is possible with spray-dried algae without significantly reducing the live (wet) growth of the clams.

Furthermore, effects of three different clays, kaolin, calcium montmorillonite and calcium bentonite, were determined. No significant difference in clam growth rate (% wet and organic weight increase) among treatments with additions of bentonite, kaolin and montmorillonite was found.

Effect of different concentrations of calcium bentonite and *Haematococcus pluvialis* were tested on the growth rate of the clams. Results indicated that different concentrations of *Haematococcus pluvialis* and calcium bentonite did not improve the growth of the animals.

Seventy five percent substitution of live algae with spray-dried algae can decrease the production cost by up to 50%. Therefore, use of spray-dried diets will greatly decrease the costs of juvenile clam nurseries and lead to more consistent and economic production.

Evaluation of Spray-dried Microalgae in Diets for Juvenile Manila Clam *Tapes philippinarum*

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INTRODUCTION

Global aquaculture production of clams, cockles and arkshells equals about 2 million tonnes per annum that is valued about 2.5 billion dollars. In 1997, 1.2 million tonnes were derived from harvests of the Manila clam *Tapes philippinarum*, with a value of about $1.5 billion. Farms in the U.S. currently produce about 2000 tonnes of Manila clams and the U.S. is the third biggest producer after China and Korea (FAO, 1999).

The traditional nursery culture of juvenile Manila clams depends on large-scale production of live microalgae. Costs of algal production are high for rearing bivalves under nursery conditions, ranging from $160 to more than $200 per kg dry weight (Coutteau and Sorgeloos, 1993). Algal production costs may represent about 30% of total spat (juvenile) production costs (Coutteau and Sorgeloos, 1992). Furthermore, production of microalgae is not reliable due to variation in food value, rapid die-offs, and bacterial contamination.

The nutritional quality of microalgal species varies due to cell size (Haven & Morales-Alamo, 1970), digestibility of the cell wall (Epifanio et *al.*, 1981) and biochemical composition of the cell contents (Webb & Chu, 1983). Different species or even different strains of the same species of microalgae may differ widely in their dietary value. For example, it has been shown by Okauchi (1991) that microalgal species

(i.e. *Nannochloropsis oculata, Pavlova lutheri, Skeletonema costratum* and *Isochrysis galbana*) cultured under the same conditions can differ in their fatty acid composition. Chemical and physical features of the algal culture environment, such as light and nutrient supply, also play an important role in determining algal dietary values. For example, when *Monodus subterraneus* is growing exponentially, it has a protein content of almost 70%. In contrast, nitrogen-deficient conditions produce *M. subterraneus* cells which have protein contents of less than 10% (Hoff and Snell, 1987). As a result of this non-reliable live algal production, there is a strong need for alternative diets to replace live microalgae as food for bivalves in nurseries. The use of artificial diets will greatly simplify hatchery-nursery procedures and lead to more consistent and economic production of bivalve spat.

Successful partial replacements for living algal diets have been reported in many different studies. Laing and Millican (1992) showed that the growth rate of *Tapes philippinarum* juveniles fed a mixture of spray-dried microalgae (70% *Tetraselmis suecica* and 30% *Cyclotella cryptica*) was not significantly different from that of juveniles fed live microalgae. Laing et al. (1990) also showed that spray-dried *Nannochloris* sp. and *Tetraselmis suecica* could support the growth of larvae of the Manila clam equal to or greater than their counterparts fed on live algae. In another study, Boeing (1997) reported that up to 40% w/w (weight/weight) of a mixed algal diet (*Tetraselmis suecica* and *Chaetoceros* sp.) could be replaced with a heterotophically-grown *Schizochytrium* sp. for Manila clams without significantly reducing their growth rate. Furthermore, he reported that the growth of clams fed *Tetraselmis suecica* alone could be improved by supplements of *Schizochytrium* sp.

A study by Curatolo et al. (1993) showed similar results to those of Boeing. Curatolo and his colleagues found that 2-3 mm Manila clam spat could be fed on an 80% live/20% dry diet of *Tetraselmis suecica* without significantly reducing the growth compared to that of clams fed on 100% live algal diet of *Chaetoceros calcitrans*. After feeding clams on the mixed diet for a month, the proportion of dry algae in the diet could be increased from 20% to 40% without adversely affecting growth.

In a study by Albentosa et al. (1989), Manila clam spat were fed on 80% yeast enriched with a growth promoting agent and 20% live algae. This mixture supported excellent growth rates, which were higher than those of spat fed on a ration composed of a mixture of living algae (*Isochrysis galbana*, *Thalassiosira pseudonana*, *Skeletonema costatum*, *Chaetoceros gracilis*, *and Tetraselmis suecica*). Another study by Coutteau et al. (1993 and 1991) revealed that yeast could support good growth of Manila clam and *Mercenaria mercenaria* juveniles. They reported that a diet composed of 80% yeast and 20% (w/w) microalgae resulted in growth rates which were 70 to 90% of those observed for clams fed on live microalgae. Moreover, replacing 50% of the living algal ration by yeast did not result in a significant decrease in the growth of *Mercenaria mercenaria* spat relative to that of controls fed on living algae (Coutteau et al., 1994).

Promising partial replacements for living algal diets have been reported for other commercially important species in addition to Manila clams. For example, in a series of experiments, Knauer and Southgate (1996) assessed the nutritional value of a spray-dried freshwater alga *Spongiococcum excentricum* for Pacific oyster (*Crassostrea gigas*) spat. They found that spray-dried *Spongiococcum excentricum* could be used as a substitute for a live algal diet at a 20% replacement level. In a study with larvae of the Blacklip Pearl

oyster *Pinctada margaritifera*, Southgate et al. (1998) showed that 50% of a mixed living algal diet (*Pavlova salina*, *Chaetoceros simplex* and *Isochrysis* sp.) could be replaced with spray-dried *Tetraselmis* without significantly affecting larval growth rate or survival. Furthermore larval growth could be improved by feeding larvae a 1:1 w/w mixture of a living algal diet and dried *Tetraselmis*.

Promising results have been obtained using micron size particles (microcapsules) as partial replacements for live microalgae fed to bivalves. When fed to juvenile *Crassostrea virginica*, microencapsulated diets supported growth rates up to 73% that of microalgae-fed controls (Langdon and Siegfried, 1984). A commercially available microncapsulated diet was used to replace 15% and 40% of a live microalgal diet for clam and oyster spat respectively, without affecting their growth rates (Laing, 1987).

Growth experiments carried by Langdon and Önal (1999) showed that juvenile mussels *Mytilus galloprovincialis* could be cultured on a spray-dried algal diet of 50% w/w *Schizochytrium* sp. and 50% w/w *Spirulina* sp. without significantly reducing growth rate compared with that of clams fed on living algae. Growth of mussels fed spray-dried diets composed of mixtures of *Schizochytrium* sp., *Spirulina* sp. or *Haematococcus pluvialis* was not significantly different from that of mussels fed an equal ration (by dry weight) of a mixed diet of living Tahitian *Isochrysis galbana* and *Chaetoceros calcitrans*. Furthermore, growth of mussels could be improved significantly by adding the spray-dried diet to a 1/4 ration of living algae.

Clay is known to have beneficial effects on growth of bivalves by increasing rates of pumping and reducing pseudofecal production. Also, addition of naturally occurring silt to algal diets is known to increase the growth of bivalves (Murken 1976; Winter,

1976; Kiørboe et al., 1981; Ali 1983; Ewart, 1983). For example, Kiørboe et al. (1981) reported that adding 5mg l^{-1} of natural silt to microalgae fed to juvenile mussel *Mytilus edulis* improved their growth by 30 to 70%. In other studies, addition of 20 mg kaolin l^{-1}, a type of clay, to artificial and yeast-based diets has also been shown to improve the growth of *Crassostrea virginica* juveniles (Langdon and Bolton, 1984; Langdon and Siegfried, 1984; Urban and Langdon, 1984). Ali (1983) found that silt supplementation of up to 25 mg l^{-1} increased the growth of oysters (*Crassostrea virginica*) fed at high algal concentrations (6×10^{-5} cell ml^{-1}).

In the present study, commercially available spray-dried *Schizochytrium* sp. and *Haematococcus pluvialis* were fed to juvenile Manila clams *Tapes philippinarum*. This clam species is a member of the family *Veneridae* and is native to the bays and protected coasts of Japan. Now it is cultured worldwide and is common from Alaska to California on the west coast of the U.S. (Manzi and Castagna, 1989). This commercially important clam species is in high demand. Supplies rarely exceed demand and a relatively high price structure is supported and maintained. Reducing the high cost of farming these animals is a research objective that could have significant commercial benefits.

Schizochytrium sp. is a unicellular thraustochytrid, which is rich in n-3 and n-6 long chain fatty acids (Barclay and Zeller, 1996) (Table 1). Thraustochytrids are a unique group of eukaryotes, completely separate from fungi, and are closely related to red and brown algae (Mannella et al., 1987). They are marine, non-photosynthetic protists, and they can be produced heterotrophicaly in large amounts by fermentation. Heterotrophic production has several potential advantages over photosynthetic production including:

1) a high degree of process control which can facilitate rapid growth and production, easy maintenance of monocultures, and consistent, reproducible products; and 2) lower costs for harvesting the biomass because of the higher cell densities normally achieved with this type of production (Barclay et al., 1994).

Haematococcus pluvialis is a unicellular alga, which belongs to the division Chlorophyta and order Volvocales. Although it is classified in the Chlamydomonadaceae family it is sometimes assigned membership in the family Haematococcaceae. It can use organic nitrogen and acetate and grow heterotrophically in darkness (Bold and Wynne, 1985). It contains astaxanthin, which gives it a red color. Astaxanthin is used as a pigment and nutritional ingredient in feeds for aquatic animals. *Haematococcus pluvialis* also contains arachidonic acid (20:4 n-6) and eicosapentaenoic acid (20:5 n-3) (pers. com. Sanders Brine Shrimp).

In this study, I report the results of growth experiments with juvenile Manila clams, *Tapes philippinarum*, in which I tested combinations of a spray-dried, *Schizochytrium* product (Docosa Gold, Sanders Brine Shrimp) and *Haematococcus pluvialis* product (Sanders Brine Shrimp) either as supplements or as complete replacements for living algae. The second objective of this study was to compare the effect of additions of different types of clay to diets on the growth and survival of juvenile Manila clams.

The purpose of the present study was to calculate the growth and survival rates of *Tapes philippinarum*, a species that is widely distributed and commercially important. This study is accomplished for the first time with *Haematococcus pluvialis* and it may help to enable the costs of juvenile clam nurseries to be decreased.

MATERIALS AND METHODS

Culture system

Feeding experiments were carried out in 10 l plastic containers (Rubbermaid; soft wastebasket, Part no: 2955-00) (Figure 1). Strong aeration (1300 ml/min.) was provided to each container to keep food and clay particles in suspension.

Culture water was first passed through a sand filter to remove large particles bigger than 50 microns then pumped through a 10, 5 and finally 1 micron cartridge filters to remove small particles. A flow-through heater (Hot Shot instantaneous water heater; Model HCT484-S-G-X) was used to heat the seawater. Seawater temperature was maintained at 19 ± 1 ^{o}C during the experiments.

Culture conditions

Culture water was changed every two to three days. Containers were cleaned with hot fresh water at each water change. Seawater salinity was measured at every water change and salinity ranged between 30-35 ppt. averaging 32 ppt. during the experiments. Each growth experiment lasted three weeks. Triplicate cultures were set up for each test diet in all experiments.

Experimental juvenile Manila clams were obtained from Taylor Shellfish Farms, Washington, U.S (Figure 2). Groups of 15 animals were placed in each container in Experiments 1, 2 and 4, while a stocking density of 10 animals per container was used in Experiment 3.

Figure 1. Culture system used for feeding experiments with Manila clams.

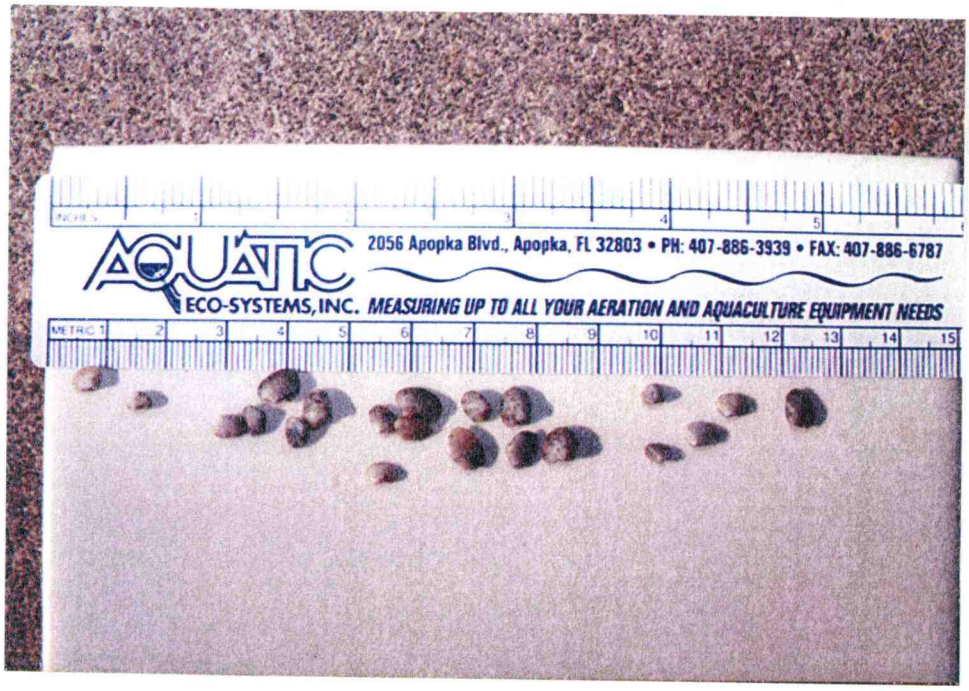

Figure 2. Juvenile Manila clams used for the feeding experiments.

Diets and rations

Animals were fed twice daily on either living microalgal diets or various non-living, spray-dried diets of *Schizochytrium* sp. or *Haematococcus pluvialis* (Figure 3 and 4). Spray-dried diets were supplied by Sanders Brine Shrimp Company, Utah, U.S (Table 1). Living algal diets were cultured at the Hatfield Marine Science Center, Oregon, U.S. Algal diets were cultured under continuous light according to methods described by Breese and Malouf (1975) and harvested during late exponential growth phase.

Table 1. Biochemical composition of spray-dried diets fed to clam *Tapes philippinarum* in this study.

Nutrient (% dry weight)	*Schizochytrium* sp.	*H. pluvialis*
Proximate composition (% dry weight)		
Protein[a]	39	25
Carbohydrate[a]	18	37
Lipid[a]	26	21
Fatty acid composition[a] (% fatty acid content)		
20:4 *n*-6	2.1	2.2
20:5 *n*-3	1.5	1.4
22:5 *n*-6	12	0.1
22:6 *n*-3	33	0
Cholesterol (% dry weight)	0.25[b]	0.067[a]

[a] Data from Sanders Brine Shrimp. [b] Data from Barclay and Zeller (1996).

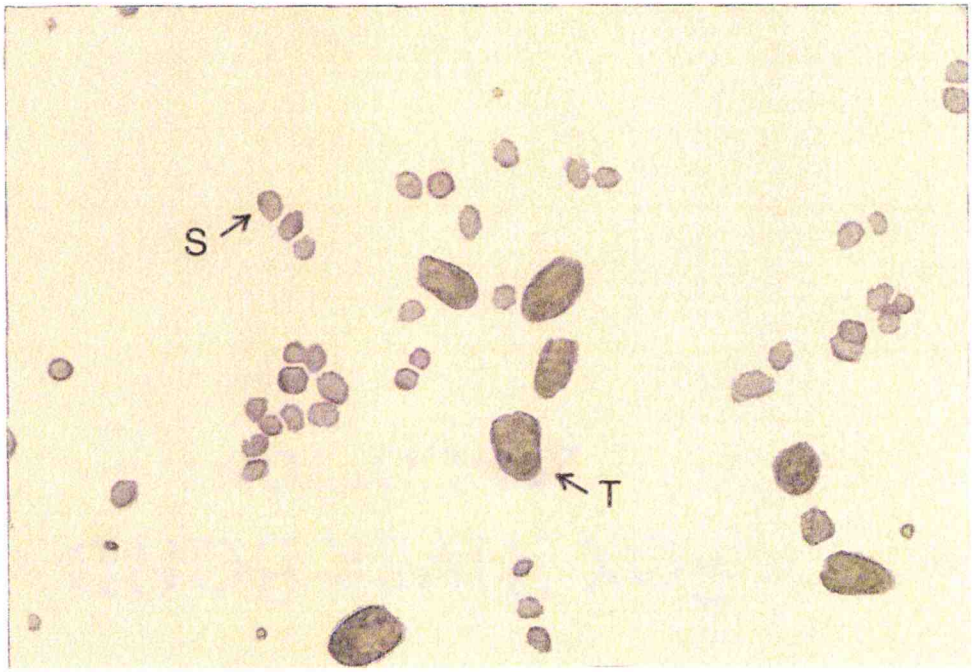

Figure 3. *Schizochytrium* sp. used for the feeding experiments. *Schizochytrium* cells are shown with *Tetraselmis* sp. cells for size comparison. Magnification, 800X. Scale; 1cm = 12.5μm. S= *Schizochytrium* sp., T= *Tetraselmis* sp.

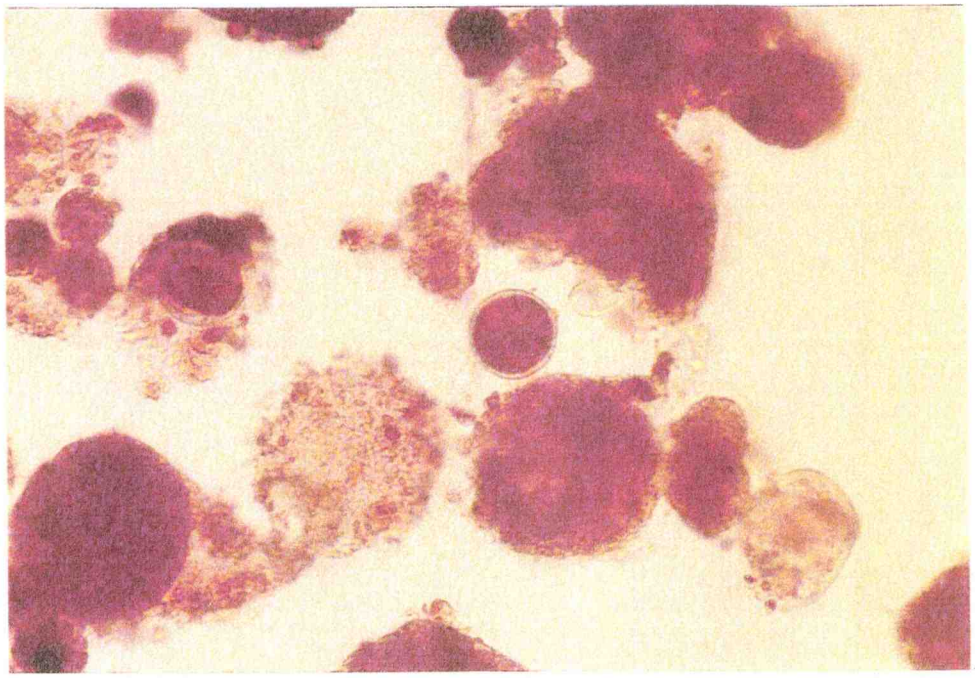

Figure 4. *Haematococcus pluvialis* used for the feeding experiments. Magnification, 500X. Scale; 1cm = 20 μm.

Stock suspensions of dry, spray-dried diets were produced by mixing aliquots of the diets with distilled freshwater. The suspensions were mixed on a magnetic stirrer then sonicated for 30 seconds to minimize clumping. The suspensions were stored at 5 ^{o}C. A full dietary ration was defined as 25 mg dry weight of diet added to each 10 l culture, resulting in a food concentration of 2.5 µg dry weight ml^{-1}. This concentration was equivalent, in terms of dry weight, to a mixed algal diet of 50,000 cells ml^{-1} Tahitian *Isochrysis galbana* (T-ISO) and 50,000 cells ml^{-1} *Chaetoceros calcitrans* (Cc), assuming a T-ISO cell weight of 2 x 10^{-8} mg and a Cc cell weight of 3 x 10^{-8} mg (Urban and Langdon, 1984; Önal per. obs.). A full ration of 2.5 µg dry weight ml^{-1} of *Schizochytrium* sp. resulted in a cell concentration of 25,000 cells ml^{-1} (Gouthro et al., 1998). Individual cell size was 5-10 microns (Sanders Brine Shrimp). A full ration of 2.5 µg dry weight ml^{-1} of *Haematococcus pluvialis* resulted in a cell concentration of 1500 cells ml^{-1} with an average individual cell size of 14 microns (Önal per. obs.).

Growth experiments

In Experiment 1, the objective was to examine if it was possible to replace live algae totally or partially with a spray-dried diet. Therefore, non-algal diets were tested either as supplements to live algae or alone. Spray-dried *Schizochytrium* sp. or *Haematococcus pluvialis* were added to different rations of live algae ranging from a full ration to 1/16 of full ration. Clams were fed twice a day on either x0, x1/4, x1/16, or x1 a full ration of a mixed algal diet consisting T-ISO and Cc. Full rations were adjusted during the experiment so that the animals would consume 50-70% of the food in the

container. Full rations were increased from 50,000 cells ml^{-1} ($125x10^{-6}$ mg/l) to 70,000 cells ml^{-1} ($175x10^{-6}$ mg/l) during the experiment, equivalent to about 2% to 1.5% of the wet weight of spat fed per day, respectively to meet increased food requirements as spat grew in size. The rations of algae and supplements were increased to the same degree as the spat grew to ensure that the ratio of food ingredients was constant throughout the experiment. Each of the algal rations was either fed alone or with supplements of full rations of spray-dried *Schizochytrium* sp.(SZ) or full rations of spray-dried *Haematococcus pluvialis* (H) (Table 2). Initial individual clam shell lengths ranged between 3 to 6 mm and average initial individual clam live weight was 37.5 ± 0.93 mg.

In Experiment 2, the effects of supplements of the two spray-dried diets were tested with a 1/16 ration of live algae. The objective was to improve the growth of the Manila clam spat. Different proportions of SZ and H, ranging from 100% SZ to 100% H, were fed to clams with or without live algae (Table 3). Full rations ranged from 50,000 cells ml^{-1} to 70,000 cells ml^{-1} during the experiment, equivalent to about 2% to 1.9% of the wet weight of spat fed per day, respectively. Clams were fed twice a day on either x0, x1/16 or x1 of a full ration of a mixed diet algal diet consisting of T-ISO and Cc. The full ration was adjusted during the experiment so that the animals would consume 50-70% of the food in the container. Initial individual clam shell lengths ranged between 4 to 6 mm and average initial individual clam live weight was 37.13 ± 1.49 mg.

In Experiment 3, the effect on clam growth of calcium bentonite additions to algal-supplemented diets was tested. The aim of this experiment was to improve the performance of clams fed on supplements by adding clay to the treatments. Clams were fed twice a day on either x0, x1/4, x1/16 or x1 of a ration of a mixed diet consisting of

12500 cells ml^{-1} T-ISO and 12500 cells ml^{-1} Cc, equivalent to about 5% to 3% of the wet weight of spat fed per day. Each of the algal rations was either fed alone or with supplements of full rations of spray-dried *Schizochytrium* sp. or spray-dried *Haematococcus pluvialis* (Table 4). The full ration was held constant (25,000 cell ml^{-1}) during the experiment. In this experiment calcium bentonite (American Colloid Ltd., Alabama) was added at 2mg/l to the treatments to test the effect on clam growth. Initial individual clam shell lengths ranged between 3 to 5 mm and average initial individual clam live weight was 11.4 ± 0.40 mg.

In experiment 4, three different dietary variables were tested: 1) the effect of additions of different clays (kaolin, calcium montmorillonite or calcium bentonite); 2) the effect of different concentrations of calcium bentonite; 3) effect of different concentrations of *Haematococcus pluvialis*.

Clams were fed twice a day on either x0, x1/4, or x1 of a full ration of a mixed algal diet consisting of 25,000 cells ml^{-1} T-ISO and 25,000 cells ml^{-1} Cc, equivalent to about 2.6% to 1.2% of the live weight of spat. The full ration was held constant (50,000 cell ml^{-1}) during the experiment. Each of these algal rations was either fed alone or with supplements of either x1/2, x1, x2 of a full ration of spray-dried *Haematococcus pluvialis* and different concentrations of clays (Table 5). In this experiment, calcium bentonite was added at 1 mg/l, 5 mg/l or 10 mg/l to the treatments. Kaolin (Wilkinson Kaolin Associates Ltd., Georgia) and calcium montmorillonite (Texas type; Clay Minerals Repository, Missouri) were added at 2mg/l to the treatments to test their effects on clam growth. Half and double rations of *Haematococcus pluvialis* were also tested as

supplements. Initial individual clam shell lengths ranged between 4 to 7 mm and average initial individual clam live weight was 32.4 ± 0.85 mg.

Determination of growth and survival

At the beginning of each experiment, clams were sieved using a 4mm sieve, to obtain clams of similar size then groups of clams were randomly picked from the sieved population. Group weights of clams (live weight) were taken and then clams were placed into each container. Initial live and organic weights of a random sample of 15 clams (Experiments 1, 2 and 4) or 10 clams (Experiment 3) from the population were also determined in triplicate for each experiment to estimate initial organic weights. At the end of each experiment, percent survival was determined and final live and organic weights were compared to initial weights. Percent increases in group live and organic weights were calculated.

Organic weights were calculated to estimate how much of the clam growth was meat growth. Organic weights were determined by first determining the dry weights of groups of clams by baking them in an oven at 60 ^{o}C for 48 hours. Then ash weights were determined by heating the groups of clams in a furnace at 450 ^{o}C for 24 hours. Ash weights were then subtracted from dry weights to determine organic group weights. Final group weights were adjusted for mortalities by assuming that if dead clams had survived to the end of the experiment they would have grown at the same rate as living clams in their group.

Statistics

Statistical analyses were carried out using the software package Statgraphics plus (version 3.1). When Cochran's C test and Bartlett's test showed that the standard deviations (variances) of wet and organic weights within each of the diets were not similar, data were log transformed. When log transformation was not successful in eliminating significant differences among variances, the square root of percent mean values were calculated and used in ANOVA. Tukey HSD multiple range tests were applied to determine significant differences among treatment means at a $P = 0.05$ level of significance ($n = 3$).

RESULTS

Supplements for full or partial rations of living algae

In the first experiment different types of spray-dried diets were compared with live algae in diets for clams. The aim of this experiment was to replace live algae totally or partially with different types of spray-dried diets (Table 2).

Clam survival was 100% in all cultures. Overall, there was a significant (ANOVA; $P< 0.0001$) improvement in clam growth rate (% wet and organic weight increase) when supplements of SZ or H were added to live algal rations, except when added to full algal rations (Figure 5). The highest percent increase in clam wet weight occurred with 1/4 ration of live algae and a supplement of H. Growth of clams fed this latter supplemented diet was not significantly different (Tukey; $P > 0.05$) from that of clams fed on a full live algal ration alone.

The highest percent increase in clam organic weight occurred with a full ration of live algae plus a supplement of H. Growth of clams fed this latter supplemented diet, however, was not significantly different (Tukey; $P > 0.05$) from that of clams fed on a full live algal ration alone. Growth (% wet and organic increase) of clams fed dietary supplements of SZ or H alone was significantly greater (Tukey; $P > 0.05$) than that of starved clams. Starved clams lost organic weight and showed little increase in wet weight.

Table 2. Dietary treatments of Experiment 1. A full ration of algae was defined as a 50/50% mixture of Tahitian *Isochrysis galbana* and *Chaetoceros calcitrans* added twice a day to a 10 l container. A full algal ration of a spray-dried diet increased from 50,000 cells ml^{-1} to 70,000 cells ml^{-1} during the experiment, equivalent to about 2% to 1.5% of the wet weight of spat fed per day, added twice a day to a 10 l container.
SZ= *Schizochytrium* sp., H= *Haematococcus pluvialis*.

Treatment	**% Wet Growth**	**% Organic Growth**
Starved	14 ± 0.6	-1 ± 7.4
100 % live algae Full Ration (FR)	166 ± 20.7	218 ± 22.5
1/4 FR	128 ± 3.7	95 ± 7
1/16 FR	50 ± 2.9	26 ± 4.9
FR + SZ	162 ± 13	213 ± 13.7
1/4 FR + SZ	151 ± 5.2	151 ± 10.1
1/16 FR + SZ	92 ± 5	85 ± 4.7
SZ	44 ± 4.5	30 ± 6.6
FR + H	179 ± 26.4	237 ± 31.6
1/4 FR + H	188 ± 3.2	162 ± 5
1/16 FR + H	113 ± 3.2	96 ± 8
H	69 ± 7.6	42 ± 6.7

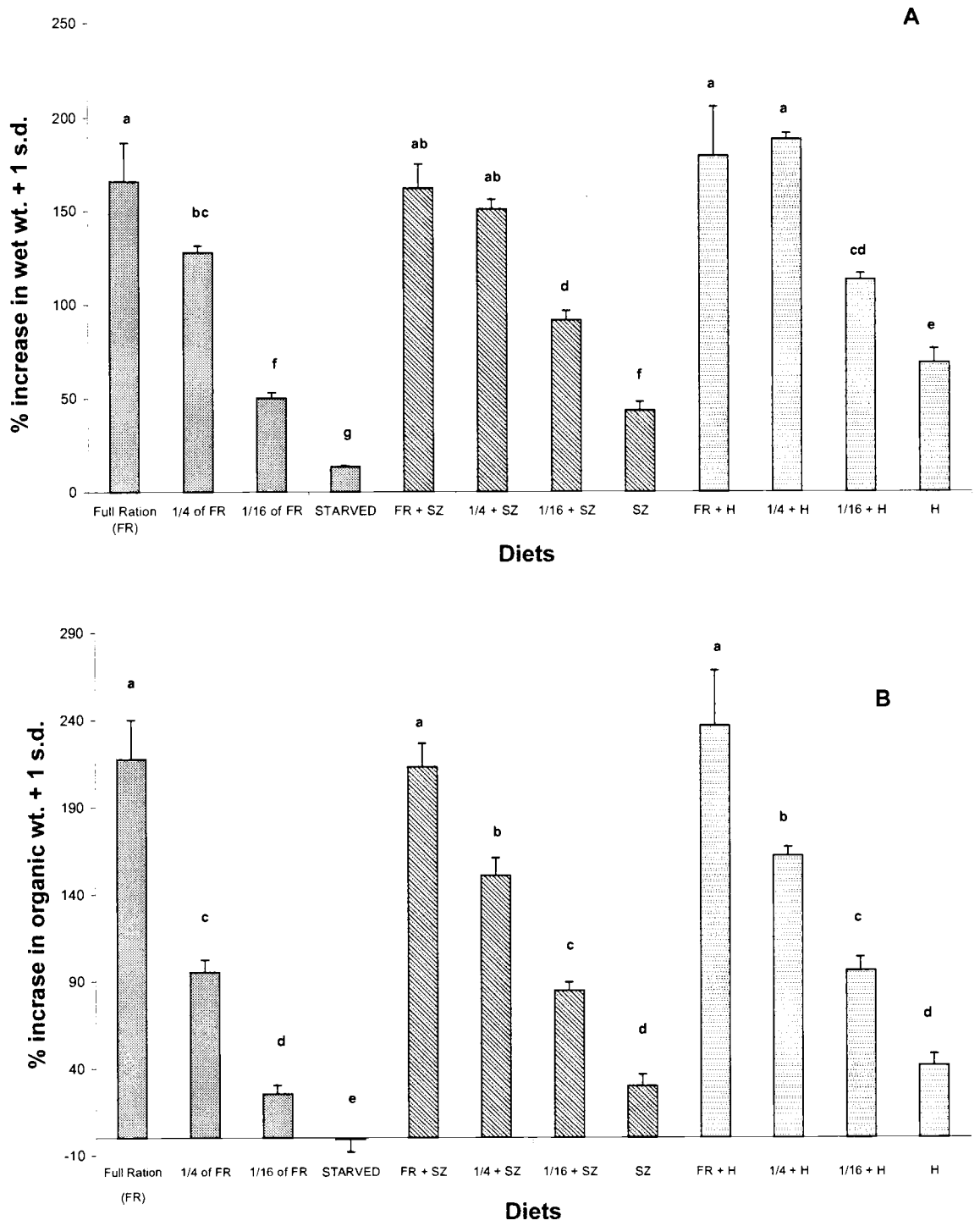

Figure 5. Growth of Manila clams *T. philippinarum* juveniles fed mixed live algal rations composed of 50% (by cell number) Tahitian *Isochrysis galbana* and 50% *Chaetoceros calcitrans* with or without supplements of spray-dried *Schizochytrium* sp. (SZ) and spray-dried *Haematococcus pluvialis* (H). A= wet (live) growth, B= organic (meat) growth.

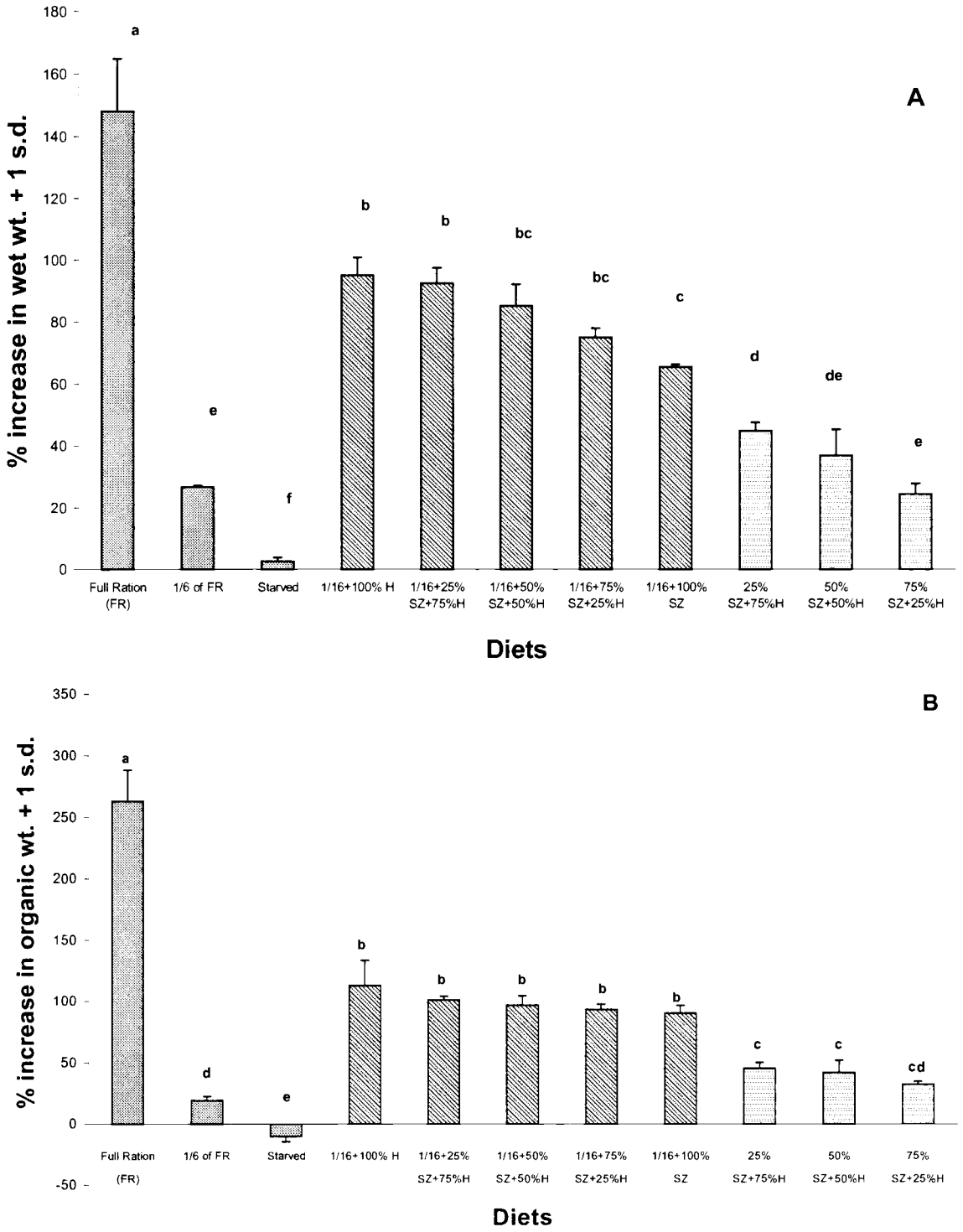

Figure 6. Growth of Manila clams *T. philippinarum* juveniles fed mixed live algal rations composed of 50% (by cell number) Tahitian *Isochrysis galbana* and 50% *Chaetoceros calcitrans* with different proportions of spray-dried *Schizochytrium* sp. (SZ) and spray-dried *Haematococcus pluvialis* (H) ranging from 0% to 100%. A= wet (live) growth, B= organic (meat) growth.

Table 3. Dietary treatments of Experiment 2. A full ration of algae was defined as a 50/50% mixture of Tahitian *Isochrysis galbana* and *Chaetoceros calcitrans* added twice a day to a 10 l container. A full algal ration of a spray-dried diet increased from 50,000 cells ml^{-1} to 70,000 cells ml^{-1} during the experiment, equivalent to about 2% to 1.9% of the wet weight of spat fed per day, added twice a day to a 10 l container.
SZ= *Schizochytrium* sp., H= *Haematococcus pluvialis*.

Treatment	% Wet Growth	% Organic Growth
Starved	3 ± 1.2	-10 ± 4.5
100 % live algae Full Ration (FR)	148 ± 16.8	263 ± 25.2
1/16 FR	27 ± 0.5	19 ± 3.2
1/16 + 100% H	95 ± 5.9	112 ± 20.5
1/16 + 75% H + 25% SZ	93 ± 4.9	100 ± 2.9
1/16 + 50% H + 50% SZ	85 ± 7.1	96 ± 7.7
1/16 + 25% H + 75% SZ	75 ± 3	93 ± 4.3
1/16 + 100% SZ	65 ± 0.8	90 ± 6.2
75% H + 25% SZ	45 ± 2.7	45 ± 4.9
50% H + 50% SZ	37 ± 8.5	41 ± 10.2
25% H + 75% SZ	24 ± 3.5	32 ± 2.9

Supplements for a 1/16 full ration of living algae

In this second experiment growth of clams fed dietary supplements of SZ and H added to a 1/16 full algal ration were determined and compared with that of clams fed a full ration of a mixed diet of T-ISO and Cc (Table 3; Figure 6).

Clam survival was 100% in all treatments. Highest wet and organic weight increase occurred when clams were fed a full live algal ration (a mixture of T-ISO and Cc). Growth (percent increase in both wet and organic weights) of clams fed a 1/16 ration of live algae supplemented with H or SZ was significantly greater (Tukey; $P< 0.0001$) than that of clams fed a 1/16 ration of live algae alone. H was better than SZ as a supplement because there was an improvement in wet and organic weight of the clams as the proportion of the H in the diet increased. Starved clams lost organic weight and showed little increase in wet weight.

Effect of calcium bentonite on clams fed on living algae with supplements of non-living feeds

The third experiment (Table 4; Figure 7) was designed to evaluate the effects of clay additions on the survival and growth of clams. Bentonite (B) was added at 2 mg/l to the treatments as it has been shown to benefit the growth of oyster spat (*Crassostrea gigas*) in preliminary experiments at Hatfield Marine Science Center.

In this experiment a total of 13 treatments were tested. Clam survival was 100% percent in all treatments except in one culture (H + B) which had a survival of 97%. The highest percent increase in clam wet weight occurred with 1/4 of a full ration of living algae supplemented with H and bentonite. The increase in wet weight of clams fed on this diet was significantly (Tukey; $P<0.0001$) greater than that of clams fed on all other rations. The growth of clams fed on a 1/16 full ration of living algae supplemented with H or SZ and bentonite was significantly (Tukey; $P<0.0001$) greater than that of clams fed

on a 1/16 full ration alone (% wet and organic weight increase). There was no significant (Tukey; $P>0.05$) difference between the wet and organic weights of clams fed SZ or H alone and that of clams fed SZ or H plus bentonite. The highest percent increase in clam organic weight occurred with 1/4 full ration supplemented with H and bentonite which was not significantly different from that of clams fed on a full ration (Tukey; $P>0.05$).

Table 4. Dietary treatments of Experiment 3. A full ration of algae was defined as a 50/50% mixture of Tahitian *Isochrysis galbana* and *Chaetoceros calcitrans* added twice a day to a 10 l container. A full algal ration of a mixed diet consisting of 12500 cells ml^{-1} T-ISO and 12500 cells ml^{-1} Cc, equivalent to about 5% to 3% of the wet weight of spat fed per day. SZ= *Schizochytrium* sp., H= *Haematococcus pluvialis*, B= calcium bentonite

Treatment	% Wet Growth	% Organic Growth	% Survival
Starved	12 ± 4.7	-7 ± 11.8	100
100 % live algae Full Ration (FR)	117 ± 25.8	183 ± 33.6	100
FR + B	120 ± 10.1	177 ± 20.5	100
1/4 FR	129 ± 4.1	134 ± 10.7	100
1/16 FR	54 ± 8.1	37 ± 5.8	100
1/4 + SZ + B	112 ± 5.2	154 ± 11.6	100
1/16 + SZ + B	90 ± 12.7	100 ± 22	100
SZ + B	52 ± 9.9	47 ± 14.2	100
SZ	41 ± 2.6	36 ± 5.2	100
1/4 + H + B	180 ± 9	185 ± 13.4	100
1/16 + H + B	107 ± 11.3	92 ± 19.9	100
H + B	48 ± 5.2	28 ± 7.6	97
H	43 ± 0.7	38 ± 1.9	100

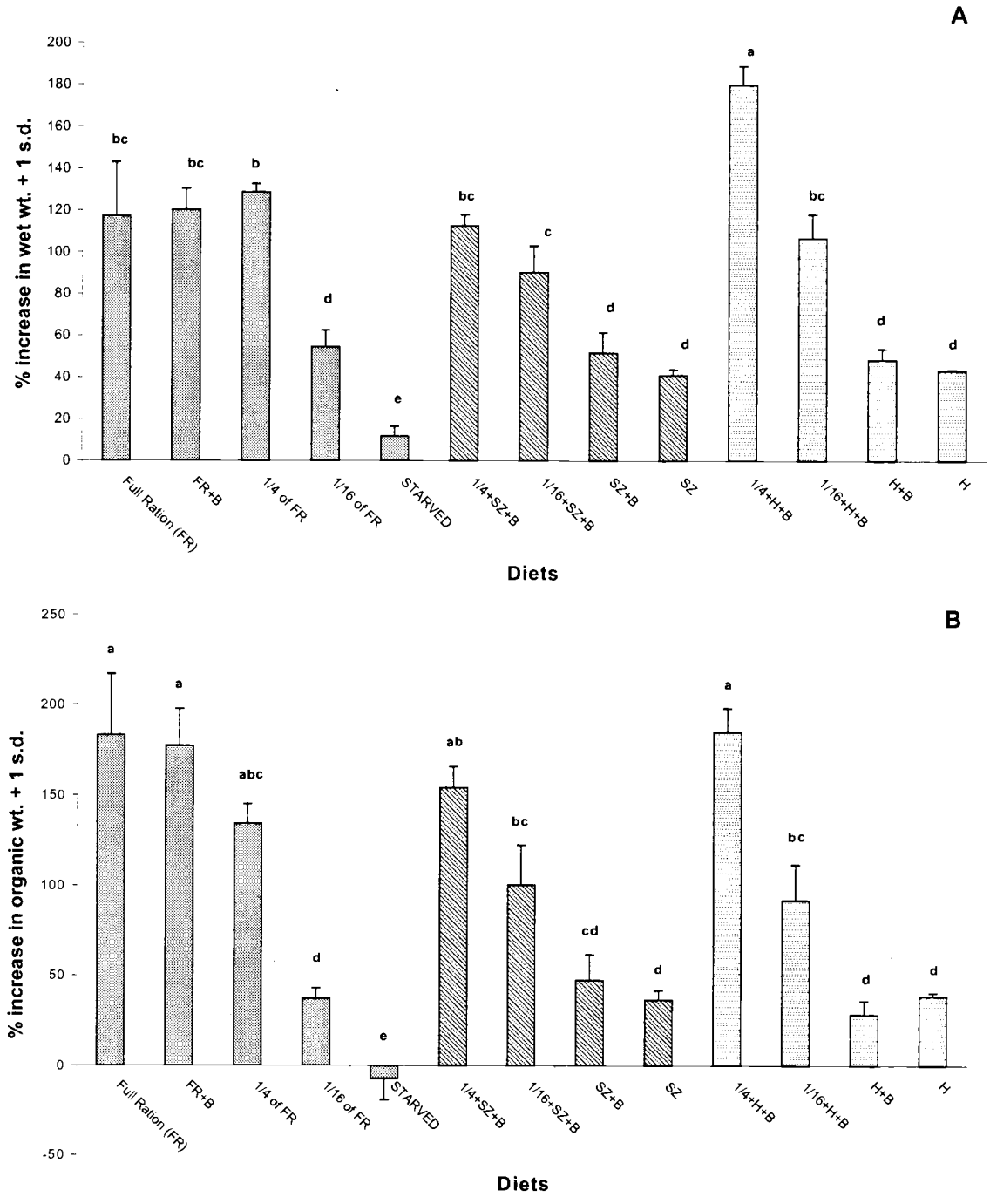

Figure 7. Growth of Manila clams *T. philippinarum* juveniles fed mixed live algal rations composed of 50% (by cell number) Tahitian *Isochrysis galbana* and 50% *Chaetoceros calcitrans* with or without supplements of spray-dried *Schizochytrium* sp. (SZ) and spray-dried *Haematococcus pluvialis* (H) and calcium bentonite (2mg/l). A= wet (live) growth, B= organic (meat) growth.

Effect of different clays on clams fed on living algae with supplements of non-living feeds

The fourth and last experiment (Table 5) was designed to evaluate effects of different types of clay such as kaolin (K), calcium montmorillonite (M) and calcium bentonite (B). In addition, different amounts of calcium bentonite and *Haematococcus pluvialis* were tested.

Clam survival was 100% percent in all cultures except two cultures (1/4 + H and 1/4 + H + 5B) which each had a survival of 98%. There was no consistent pattern of survival among the tested dietary treatments.

Overall, there was no significant difference in clam growth rate (% wet and organic weight increase) among treatments with additions of bentonite, kaolin and montmorillonite (Tukey; $P>0.05$; Figure 8). The clams fed 1/4 ration of live algae, a supplement of H and either bentonite, kaolin or montmorillonite grew slightly better (wet weight) than clams fed full rations of live algae but there was no significant difference among the treatments (Tukey; $P>0.05$). Clams fed 1/4 ration of live algae, a supplement of H and either bentonite, kaolin or montmorillonite grew slightly less (organic weight) than the clams fed full rations of live algae but again there was no significant difference among the treatments (Tukey; $P>0.05$). Calcium bentonite had no significant effect when added to 1/4 ration of live algae and a supplement of H (Tukey; $P>0.05$; Figure 8).

There was no significant difference in clam growth rate (% wet and organic weight increase) among treatments with different concentrations of calcium bentonite (Tukey; $P>0.05$; Figure 9). Clams fed 1/4 ration of live algae and a supplement of H and different concentrations of bentonite grew slightly better than clams fed on the same diet

Table 5. Dietary treatments of Experiment 4. A full ration of algae was defined as a 50/50% mixture of Tahitian *Isochrysis galbana* and *Chaetoceros calcitrans* added twice a day to a 10 l container. A full algal ration of a mixed algal diet consisting of 25,000 cells ml^{-1} T-ISO and 25,000 cells ml^{-1} Cc, equivalent to about 2.6% to 1.2% of the wet weight of spat, fed per day. H= *Haematococcus pluvialis*, B= calcium bentonite, K= kaolin, M= montmorillonite. B, K and M were added at 2mg/L twice a day. 1/2B= 1mg/L, 5/2B= 5mg/L and 5B= 10mg/L.

Treatment	% Wet Growth	% Organic Growth	% Survival
Starved	5 ± 0.9	7 ± 6.3	100
100 % live algae Full Ration (FR)	132 ± 21.9	244 ± 43.7	100
1/4 FR	88 ± 4.4	138 ± 34.3	100
1/4 + H	140 ± 9.3	166 ± 6.9	98
1/4 + H + B	145 ± 12.1	169 ± 13.8	100
1/4 + H + K	145 ± 15.4	167 ± 3.2	100
1/4 + H + M	145 ± 13.5	170 ± 11.4	100
1/4 + H + 1/2B	130 ± 3.1	166 ± 18.5	100
1/4 + H + 5/2B	132 ± 8.5	172 ± 3.6	100
1/4 + H + 5B	132 ± 4.4	161 ± 6.3	98
1/4 + 1/2H + B	124 ± 14.8	138 ± 5.6	100
1/4 + 2H + B	131 ± 13.8	178 ± 24.5	100

without clay but there was no significant difference among the treatments (Tukey; $P>0.05$). Growth rates of clams (% increase in wet weight) fed a 1/4 ration of live algae, a supplement of H and various amounts of bentonite were not significantly different from that of clams fed a full ration of live algae (Tukey; $P>0.05$).

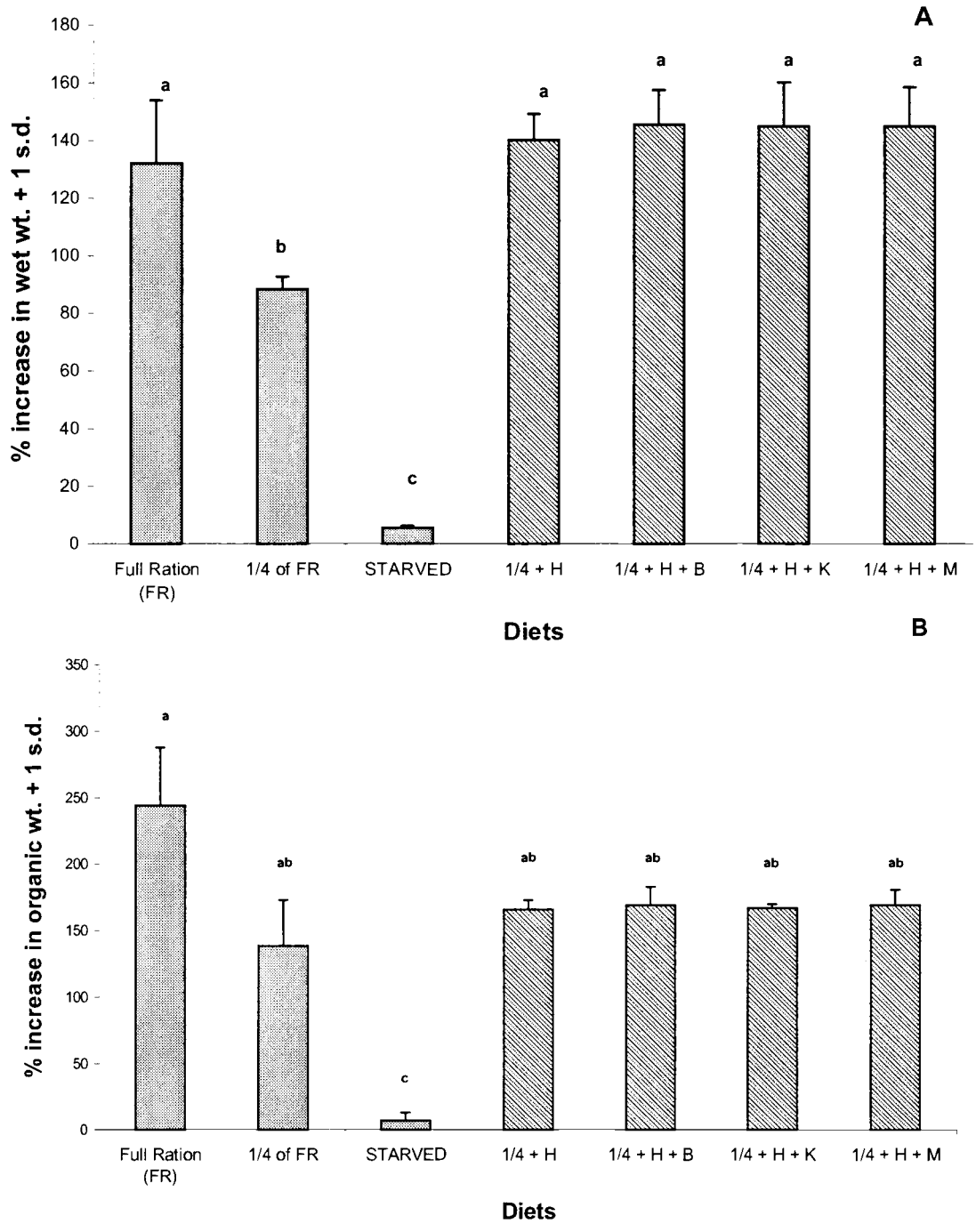

Figure 8. Growth of Manila clams *T. philippinarum* juveniles fed mixed live algal rations composed of 50% (by cell number) Tahitian *Isochrysis galbana* and 50% *Chaetoceros calcitrans* with a supplement of spray-dried *Haematococcus pluvialis* (H) and different types of clays (2mg/l). A= wet (live) growth, B= organic (meat) growth.

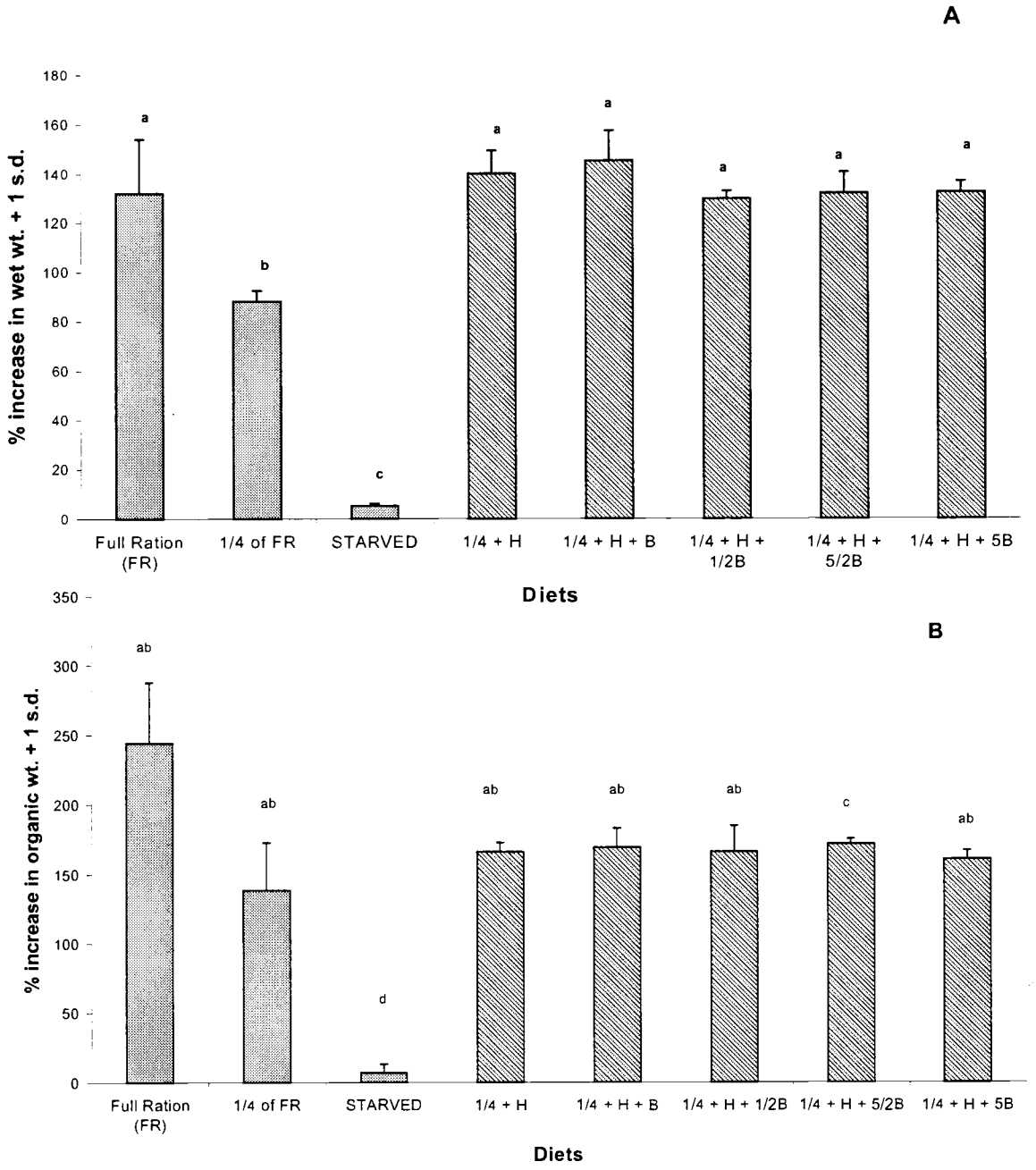

Figure 9. Growth of Manila clams *T. philippinarum* juveniles fed mixed live algal rations composed of 50% (by cell number) Tahitian Isochrysis galbana and 50% Chaetoceros calcitrans with a supplement of spray-dried *Haematococcus pluvialis* (H) and different concentrations of calcium bentonite. B= 2mg/l, 1/2B= 1mg/l, 5/2B= 5mg/l and 5B= 10mg/l. A= wet (live) growth, B= organic (meat) growth.

Organic growth rates of clams fed a 1/4 ration of live algae, a supplement of H and various concentrations of bentonite were not significantly different from that of clams fed a full ration of live algae, except for the growth of clams in the treatment with additions of the mid concentration of 5mg/l bentonite. The organic growth of clams in the presence of additions of 5 mg/l bentonite was significantly less than that of clams fed on a full ration of live algae (Tukey; $P<0.005$).

There was no significant difference in clam growth rate (% wet and organic weight increase) due to additions of different amounts of H to a 1/4 ration of algae (Tukey; $P>0.05$; Figure 10). Clams fed on a 1/4 ration of live algae with various amounts of H and bentonite grew at similar rates to clams fed a full ration of live algae (Tukey; $P>0.05$). Starved clams showed little increase in wet and organic weight during the experiment.

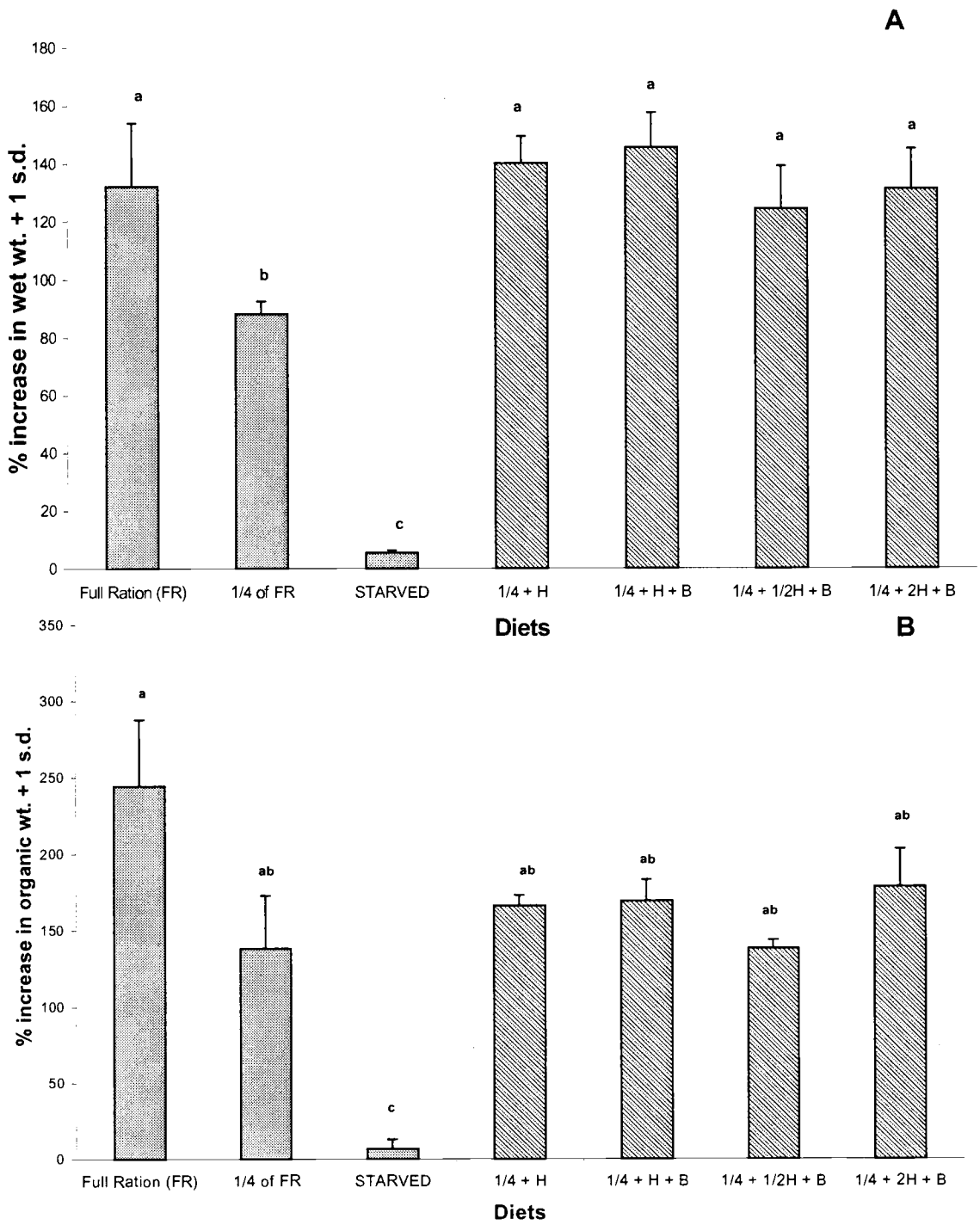

Figure 10. Growth of Manila clams *T. philippinarum* juveniles fed mixed live algal rations composed of 50% (by cell number) Tahitian *Isochrysis galbana* and 50%*Chaetoceros calcitrans* with different concentrations of spray-dried *Haematococcus pluvialis* (H) and calcium bentonite (2mg/l). A= wet (live) growth, B= organic (meat) growth.

DISCUSSION

Supplements for partial rations of living algae

Results of the present study showed that two different spray-dried diets supported clam growth when added as supplements to rations of a mixed diet of *I. galbana* and *C. calcitrans*. There was a significant (ANOVA; $P< 0.0001$) improvement in clam growth rate (% wet and organic weight increase) when supplements of *Schizochytrium* sp. or *H. pluvialis* (1.25×10^{-3} g/l) were added to partial rations of live algae but not when added to full algal rations. Growth (wet wt. increase) of clams fed on 1/4 ration of live algae and a supplement of *H. pluvialis* or *Schizochytrium* sp. was similar to that of clams fed on a full live algal ration alone (Tukey; $P > 0.05$). This result shows that 75% substitution of live algae was possible with spray-dried algae without significantly reducing clam growth (wet wt. increase).

In contrast with this study, Curatolo et al. (1993) found that only 40% of a ration of live *C. calcitrans* could be substituted with dried *Tetraselmis suecica* without a reduction in growth, compared to that of Manila clams fed entirely live *C. calcitrans*. The lower substitution level with dried *T. suecica* compared with *H. pluvialis* or *Schizochytrium* sp. may be due to differences in the composition of the spray-dried diets. Total lipid content (% organic weight) of dried *T. suecica* was 5% whereas the total lipid contents of *Schizochytrium* sp. and *H. pluvialis* were 26% and 21%, respectively. Curatolo et al. also indicated that bacterial contamination associated with dried algal diets resulted in negative growth rates. However Laing and Verdugo (1991) reported that dried

T. suecica was a good substitute for living algae. They found that Manila clam spat fed on a 20% ration of *C. calcitrans* and spray-dried *T. suecica* grew significantly better than spat fed on a 100% ration of live *C. calcitrans* alone. Laing and Millican (1992) also found that Manila clams fed on a mixture of 10% live *Skeletonema costatum* and 90% dried *T. suecica* grew as well as with a live mixed algal diet of *T. suecica* and *S. costatum*.

I found that the growth of Manila clams fed spray-dried *Schizochytrium* sp. or *H. pluvialis* alone was not satisfactory. *H. pluvialis* alone supported better growth than a 1/16 ration of live algae but poorer than a 1/4 live algal ration. It was impossible to improve the growth of Manila clams fed *H. pluvialis* by mixing *H. pluvialis* with different proportions of *Schizochytrium* sp. In contrast, Laing and Millican (1992) reported that clams fed a ration composed of 100% dried algae (70% *T. suecica* and 30% *Cyclotella cryptica*) grew almost as well as with a ration made up of 100% live algae (70% *T. suecica* and 30% *S. costatum*). It is possible that the dried diet used by Laing and Millican could satisfy the dietary requirements of the clams better than the dried diets or the live *T. suecica* and 30% *S. costatum* was not as nutritious as *C. calcitrans* and *I. galbana* used in this study.

Effect of different clays on clams fed on living algae with supplements of non-living feeds

The Manila clam is a good candidate for feeding experiments because of its ability to use a variety of food sources. Clams can satisfy their nutritive needs from detritus, dissolved compounds, and microalgae (Jogersen, 1966). Enzymatic studies have

shown that the digestive diverticula of clams contain cellulases for digestion of detritus (Sharp et al., 1985). Clams also dwell in silty sand; therefore it is possible that they take up silt with algae during feeding.

In this study three different clays, kaolin (K), calcium montmorillonite (M) and calcium bentonite (B), were used in an attempt to improve the growth of clams fed on dried algae. There was no significant difference in clam growth rate (% change in live or organic weights) among treatments with additions of bentonite, kaolin or montmorillonite (Tukey; $P>0.05$). Clams fed on a 1/4 ration of live algae, a supplement of *H. pluvialis* and either bentonite, kaolin or montmorillonite grew slightly better (wet weight increase) than clams fed full rations of live algae but there was no significant difference among these treatments (Tukey; $P>0.05$). Growth of clams fed on a 1/4 ration of live algae and a supplement of *H. pluvialis* was not significantly different from that of clams fed on this diet with additions of clay (Tukey; $P>0.05$). Knauer and Southgate (1996) also reported that addition of 5 or 20 mg/l kaolin to spray-dried *Spongiococcum excentricum* did not result in any significant improvement in the growth of *Crassostrea gigas* spat, supporting the results of this study. In contrast Kiørboe et al. (1981), Ewart (1983) and Ali (1983) reported positive effects of additions of clays on growth of bivalves. For example, Kiørboe et al. (1981) reported that adding 5 mg/l of natural silt to microalgae fed to *Mytilus edulis* juveniles improved their growth by 30 to 70%. The positive effect of clay on the growth of bivalves could be either due to increased breakdown of the ingested diet (mechanical abrasion of ingested cells) or due to delivery of dissolved organic matter adsorbed onto the surface of clay particles. Although clay particles may have increased

nutrient availability in this study, it was not helpful in improving clam growth perhaps due to limiting essential nutrients of the spray-dried diets.

Effect of different concentrations of calcium bentonite and *H. pluvialis* on clams

I also tested the effect of different concentrations of calcium bentonite and *H. pluvialis* (H) on the growth of clams. There was no significant difference among clam live growth rates at different concentrations of calcium bentonite (B) (Tukey; $P>0.05$). A full ration of B equaled 2mg/l. Treatments of 5/2B (5mg/L) and 5B (10 mg/L) were also tested. Clams fed on a 1/4 ration of live algae with *H. pluvialis* grew at similar rates to clams fed on a 1/4 ration of live algae with either H and B, H + 1/2B, H + 5/2B or H + 5B. Similar results were obtained for organic growth except that additions of 5 mg/l bentonite (5/2B) to 1/4 algal ration H resulted in significantly less organic growth than that of clams fed on 1/4 algal ration + H alone.

There were no significant differences in clam growth rate (% change in wet or organic weights) due to the addition of different supplements of *H. pluvialis* to 1/4 ration of live algae (Tukey; $P>0.05$). Clams fed on a 1/4 ration of live algae with different additions of *H. pluvialis* (ranging between 50% full ration and 200% full ration) and with bentonite (2mg/l) grew at similar rates to clams fed on a full ration of live algae (Tukey; $P>0.05$). This result shows that by using as little as a supplement of 50% full ration of *H. pluvialis* (0.625g/l) added to a 1/4 algal ration it is possible to obtain the same growth rate as with a full ration of live algae.

CONCLUSION

Langdon and Önal (1999) were able to grow mussels successfully on mixtures of dried *Schizochytrium* sp. and dried *Spirulina* sp. without reducing mussel growth compared to that of controls fed on full rations of live algae. It is possible that the digestive capabilities and/or dietary requirements of clams are different from those of mussels. Mussels may contain a more diverse suite of digestive enzymes which helps them digest a greater variety of food types, thus, they may be more efficient than clams in digesting dried algae. Figures 11 and 12 show the feces of clams fed on dried diets. Feces containing *Schizochytrium* sp. clearly show some unbroken cells. If the clams were not able to physically breakdown the walls of *Schizochytrium* sp., it is likely that they could not efficiently use the nutrients inside the cells.

Spray-dried diets are produced by a heat drying process (up to 200 °C). Some vitamins may be lost when they are exposed to high temperatures. Also, the walls of spray-dried cells are likely to become leaky after the heating process, resulting in rapid losses of low molecular weight, water-soluble nutrients after suspension in seawater. Leakage losses of vitamins and other water-soluble nutrients from spray-dried algae could significantly reduce their nutritional value for Manila clams.

Production of the spray-dried *H. pluvialis* involved breakdown of the cell walls to assure a better bioavailability of the natural astaxanthin in feeds for aquaculture species. This lack of wall integrity will have resulted in rapid leakage of nutrients from cells suspended in seawater. Leakage of essential nutrients from the broken cells into the water

may explain the poor meat growth of clams fed on *H. pluvialis*. Shell growth of clams appeared to be less negatively affected than meat growth.

Dietary requirements of clams also may be different from those of mussels. High protein and fatty acid contents of the spray-dried *H. pluvialis* and *Schizochytrium* sp. may have been responsible for the high growth rates of mussels. However, deficiencies in other essential dietary nutrients may have limited clam growth.

Caers et al. (1998) reported that supplements of the fatty acid DHA (22:6*n*-3) added to live algae did not improve the growth of Manila clam spat fed on *Tetraselmis suecica*, which contained the fatty acid EPA (20:5*n*-3) but only trace amounts of DHA. Laing et al. (1990) proposed that Manila clams can produce DHA (22:6*n*-3) by chain elongation and desaturation from precursors of shorter chainlength, such as linolenic acid (18:3*n*-3). Therefore, Manila clams may not be dependent on DHA in their diet. This argument appears to be in agreement with the results obtained in this study. Manila clam spat grew better with spray-dried *H. pluvialis* than *Schizochytrium* sp. which contain 0% and 33% DHA, respectively. The high DHA content of *Schizochytrium* sp. may be responsible for the good growth of mussel juveniles fed on this diet while it does not seem to beneficial for Manila clam spat.

The cost of spray-dried algae ranges from $25 to $73 per kg (Sanders Brine Shrimp) whereas the cost of live algae ranges from $160 to $200 per kg dry weight (Coutteau and Sorgeloos, 1993). In this study a ration of 2.07g live algae (dry weight) was needed to produce 1g of clam meat (organic weight). The cost of producing 1g of clam meat with a ration of 100% live algae is 37 cents, however it costs only 19 cents to produce 1g of clam meat if 75% of live algae is substituted with spray-dried algae.

Seventy five percent substitution of live algae with spray-dried algae can decrease the production cost by up to 50% (Table 6). Therefore, use of spray-dried diets will greatly decrease the costs of juvenile clam nurseries and lead to more consistent and economic production.

Table 6. Average cost of live and dried algal rations to produce 1g (organic wt.) of Manila clam spat. 100% dry algal ration= 1.25mg dry wt/l.

Cost in $ to produce 1g (organic wt.) Manila clam spat	**100% live algae**	**75% live algae + 100% dry algae**	**50% live algae +100% dry algae**	**25% live algae +100% dry algae**
Live algae	0.37 [b]	0.28	0.19	0.09
Dried algae	0.0	0.10 [a]	0.10	0.10
Total cost	0.37	0.38	0.29	0.19

[a] Data from Sanders Brine Shrimp. [b] Coutteau and Sorgeloos, 1993.

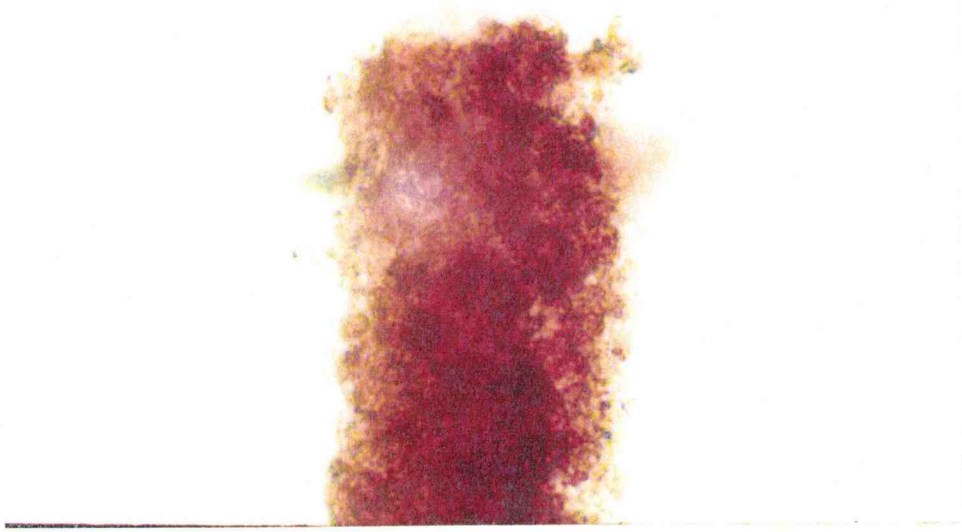

Figure 11. Feces of a Manila clam spat fed on *Haematococcus pluvialis*. Magnification, 800X. Scale; 1cm = 12.5μm.

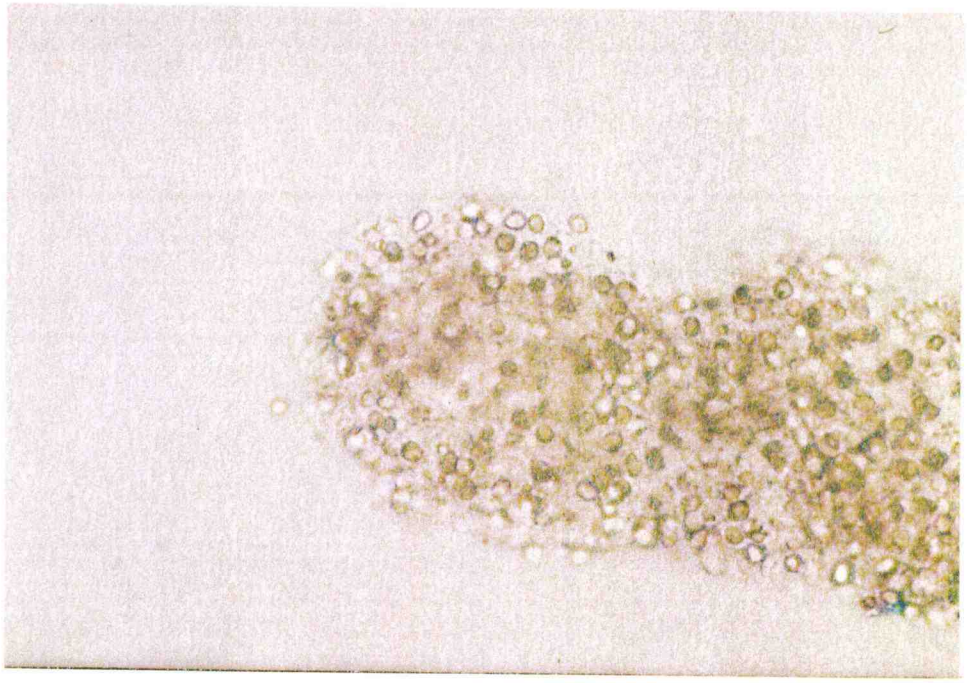

Figure 12. Feces of a Manila clam spat fed on *Schizochytrium* sp. Magnification, 500X. Scale; 1cm = 20 μm.

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