Journal of Shellfish Research, Vol. 8, No. 2, 399–405, 1989.

FOOD VALUE OF EURYTOPIC MICROALGAE TO BIVALVE LARVAE OF *CYRTOPLEURA COSTATA* (LINNAEUS, 1758), *CRASSOSTREA VIRGINICA* (GMELIN, 1791) AND *MERCENARIA MERCENARIA* (LINNAEUS, 1758)

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ABSTRACT Food values of eurytopic microalgae obtained from Solar Energy Research institute were examined by measuring larval development and growth of the bivalve species *Crassostrea virginica* (American oyster), *Cyrtopleura costata* (angel wing clam) and *Mercenaria mercenaria* (hard clam). Unialgal batch cultures of microalgae *Ellipsoidon* sp. (strain Ellip1), *Nannochloris* sp. (strain Nanno2), *Chaetoceros muelleri* Lemmermann (strain Chaet14) and *Isochrysis* aff. *galbana* Green (strain T-Iso) were grown at 30°C using 30 ppt salinity ocean water enriched with f/2 medium, silicate and urea. Larval feeding experiments were conducted in 30°C and 25 ppt salinity water. Shell length (growth) varied depending on the type of diet offered to the larvae. Whereas the number of metamorphosed larvae seems positively correlated with shell length, percent survival was not. Among the unialgal diets, *I.* aff. *galbana* and *Ellipsoidon* sp. were comparable to each other in supporting the greatest growth in *M. mercenaria* and *C. virginica*. However, *I.* aff. *galbana* supported better growth of *C. costata* larvae than *Ellipsoidon* sp. *Isochrysis.* aff. *galbana* and *Ellipsoidon* sp. were the only unialgal diets that supported metamorphosis in these bivalves within the experimental period. *Chaetoceros muelleri* and *Nannochloris* sp. could be used as feeds for larvae of these three bivalve species only when combined with other microalgae. The mixed diet consisting of equal numbers of the species *C. muelleri, Ellipsoidon* sp. and *I.* aff. *galbana* was found to be of greatest value, based on its overall capacity to support growth and metamorphosis of larvae of all three bivalve species. Moreover, its capacity to promote larval development may be attributed to balanced nutrients as suggested by the combined chemical composition.

KEYWORDS: Food value, food chemistry, microalgae, *Isochrysis* aff. *galbana, Nannochloris, Ellipsoidon, Chaetoceros muelleri, Cyrtopleura costata, Crassostrea virginica, Mercenaria Mercenaria,* bivalve larvae

INTRODUCTION

Since unicellular microalgae were recognized as a food source for bivalve larvae about half a century ago (Cole 1937, Bruce et al., 1939), few algal species have been shown to support complete larval development in pelecypods. One of the best diets discovered was *Isochrysis galbana* Parke. Unfortunately, *I. galbana* among other temperate microalgae is intolerant of high culture temperatures (Ukeles 1961). Loss of viability at 27°C has been reported in *I. galbana* by Ukeles (1961). Therefore, its usefulness is limited in tropical or subtropical hatcheries.

As interest in mariculture in tropical and subtropical regions grew, scientists obtained *Isochrysis galbana* and other temperate strains for trials with tropical molluscs. Laboratory studies showed their suitability as feeds for tropical molluscan larvae and juveniles. It was not until commercial hatchery operations were commenced that the problem of temperature tolerance with these strains emerged. Tropical hatcheries operate in warmer climates of the world. Typically, temperatures in such hatcheries vary seasonally between 14–40°C. Temperature-related problems are pronounced in summer months, where temperatures can range from 26–44°C.

To date, *Isochrysis* aff. *galbana* clone T-Iso is the only warm-water adapted phytoplankton strain available to many tropical hatcheries. This strain has been found to be a good food source for some molluscs such as *Mercenaria mercenaria* L. and *Tapes semidecussata* Reeve, but was less than satisfactory for oysters, *Crassostrea gigas* Thunberg and *C. rhizophorae* Guilding (Helm and Laing 1987). It appears obvious that the availability of phytoplankton strains for use in tropical hatcheries needs to be increased.

The Solar Energy Research Institute is engaged in fuel production from microalgae and has identified and compiled a list of microalgal strains that have a high energy yield, and a wide range of pH, temperature, salinity and light intensity tolerances (Ewart and Pruder 1981, Barclay et al. 1986, 1987, Carlson et al. 1986). Although such eurytopic microalgae were primarily selected for their potential use in biomass fuel production, they are also potentially valuable food sources either as alternatives or supplements to temperate microalgae in tropical or subtropical hatcheries. This manuscript reports the food values of four microalgal species (Solar Energy Research Institute collection) fed individually or in mixtures to larvae of three species of bivalves under tropical experimental conditions (i.e. both microalgae and larvae were cultivated at 30°C). The microalgal species tested were *Chaetoceros muelleri* Lemmermann (strain Chaet14), *Ellipsoidon* sp. (strain Ellip1), *Isochrysis* aff. *galbana* Green (strain T-Iso) and *Nannochloris* sp. (strain Nanno2). The bivalve species used

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in the feeding experiments were the American oyster *Crassostrea virginica* Gmelin, the angel wing clam *Cyrtopleura costata* L. and the hard clam *Mercenaria mercenaria* L.

MATERIALS AND METHOD

Algae

Ten microalgal strains, ranging in cell size from 4 to 10 μm (maximum linear dimension), were obtained from the Solar Energy Research Institute microalgae collection. Unialgal batch cultures of these microalgae were grown in Fernbach flasks filled with 1.5 L of sterile (autoclaved for 25 minutes at 121°C and 1.05 kg/cm^2 pressure) ocean water of 30 ± 1 ppt salinity. The ocean water was collected from the Atlantic Ocean fronting the Florida Institute of Technology Vero Laboratory, Florida. The ocean water was enriched with f/2 medium, silicate (Guillard and Ryther 1962, Guillard 1975) and urea (final concentration = 0.05 mg urea in 1 L culture water). Culture temperature was maintained at 30 ± 1°C using an incubator. Cultures were illuminated (photosynthetic photon flux fluence rate = 2.0 × 10^{15} − 4.4 × 10^{15} quanta/s-cm^2) using six Sylvania F/15T12/CW fluorescent bulbs on a 14th light: 10h dark cycle. Pairs of fluorescent bulbs were placed about 30 cm apart vertically in the incubator.

Microalgae grown in Fernbach flasks were used in preliminary feeding experiments with larval *Mercenaria* (Tan Tiu and Vaughan 1988) if they attained a density of one million cells per ml in eight days and were either motile or easily resuspended in the water column after mechanical agitation. Microalgal strains that allowed metamorphosis to occur in *Mercenaria* (Tan Tiu and Vaughan 1988) were used in feeding experiments reported here. The strains and dimensions of the microalgae, whose food values were investigated for three species of bivalve larvae are: *Chaetoceros muelleri* Lemmermann (Chaet14, 6 × 4 μm), *Ellipsoidon* sp. (Ellipp1, 4–8 × 2 μm), *Isochrysis* aff. *galbana* Green (T-Iso, 7–5 × 4 μm) and *Nannochloris* sp. (Nanno2, 4 μm).

For analyses of gross biochemical constituents, microalgae were cultured in duplicate Fernbach flasks as described above, centrifuged at 6,000 g for 10 minutes (4.0°C), rinsed and resuspended in 3.2% (w/v) aqueous ammonium formate. The resuspended cells were rapidly frozen in a dry-ice acetone bath and either lyophilized immediately or stored frozen at −40°C. Total cellular carbohydrate was determined using the modified phenol-sulfuric acid method with glycogen as a standard (Dubois et al. 1956). Cellular protein was determined by the method of Lowry et al. (1951) using a bovine serum albumin standard. Ash content was determined by combusting pre-dried microalgae (dried to constant weight at 60°C) at 550°C for 2 h. Such combustion temperature and duration was empirically determined to be within optimal range. Triplicate samples of *Chaetoceros muelleri* combusted at 30 min intervals for 4 h indicated no appreciable change in ash free dry weight from 2 to 4 h.

The nutritional component estimated by subtracting the sum of protein and carbohydrate contents from the ash free dry weight was presumed to be lipid. Protein, lipid and carbohydrate contents were expressed as proportions (%) of dry weight. The equivalent calories of the three nutritional components were calculated using the factors 5.65, 4.10 and 9.45 cal/mg for protein, carbohydrate and lipid respectively (Crisp 1971) and expressed per million cells. Values of chemical composition of mixed diets were calculated from those of unialgal diets.

Bivalves

Adult bivalves were collected from various Indian River Lagoon habitats in Florida. The angel wing clams, *Cyrtopleura costata,* were collected from Cook Point, St. Lucie County, whereas, the American oysters, *Crassostrea virginica,* and the hard clams *Mercenaria mercenaria* and *M. m. notata* Say, were harvested from Harbor Branch Oceanographic Institution cultivated clams in Sebastian, Indian River County. Some *C. virginica* were also collected from Link Port, St. Lucie County. The bivalves were spawned on the day of collection or the following day. The bivalves were induced to spawn by fluctuating temperature (22° to 29°C) or addition of sperm suspension according to the method described by Loosanoff and Davis (1963). Spawning male and female bivalves were transferred into separate containers. Eggs of several females were thoroughly mixed and fertilized by sperm from several males. In hard clams, eggs of *M. mercenaria* were fertilized by sperm from *M. m. notata* (Chanley 1961). About 100,000 to 200,000 fertilized eggs were placed in each of three Nalgene cylindrical vessels containing 15 L aerated and filtered Indian River Lagoon water of 25 ± 1 ppt salinity and 30 ± 1°C (density = 6–13 eggs/ml). Larvae were allowed to develop for 22 ± 1 h into straight-hinge stage before starting the feeding experiments. All larval culture water was maintained at 30 ± 1°C using a water bath, and illuminated with Sylvania F/15T24/CW fluorescent bulbs (photosynthetic photon flux fluence rate = 3.0 × 10^{14} − 4.0 × 10^{14} quanta/s · cm^2) on a 12 h light: 12 h dark cycle. All seawater used was ultimately filtered using a Millipore filter of 0.45 μm pore diameter after prefiltration that included a charcoal filter and exposure to uv light.

Feeding experiments were conducted in 1 L Nalgene beakers. Each treatment was run in quadruplicate. Larval density in each beaker was adjusted to 800 larvae per 800 ml (density = 1 larva/ml) of filtered but unaerated water of 25 ± 1 ppt salinity and 30 ± 1°C. The microalgae used in the feeding experiments were harvested at stationary growth phase and cell density was determined using a haemacytometer. The amount fed was adjusted initially to 25,000 cells per ml of larval culture water (25,000 cells/larva). Algal species were offered to the larvae either as a

unialgal diet or as a mixed diet once every two days from day 1 to day 9 for *M. mercenaria,* and day 1 to day 17 in *C. virginica* and *C. costata,* after the complete renewal of larval culture water. The mixed diet consisted of equal proportions, based on cell number, of its algal components at a total concentration of 25,000 cells/ml. Non-fed larvae as well as larvae fed with *Isochrysis* aff. *galbana* were run with other treatments in all feeding experiments.

One hundred to 200 larvae were sampled from each well-mixed replicate, prior to water renewal, once every two days for *Mercenaria mercenaria* or once every four days for *Crassostrea virginica* and *Cyrtopleura costata.* The number of surviving larvae was counted, and the maximum anterior posterior lengths of 30 larvae were measured for each of the four replicates. Only the data of the final sampling dates are presented here. The final average shell lengths (= growth) of each treatment for each experiment were compared by Model I ANOVA. If the ANOVA were significant, Student-Newman-Keuls (SNK) Tests were carried out on the data. F and Q statistics were evaluated at the 0.05 significance level. The percentage survival of each treatment for each experiment was likewise compared statistically (as in shell length data) after arcsine transformation. For experiment CC2, one of two experiments using *C. costata,* data on the 9th day were used instead of the final sampling date because of ciliate contamination on the latter date.

The metamorphosed larvae of *C. virginica* and *C. costata* were detected by their dissoconch shell. The numbers of metamorphosed larvae in *C. virginica* and *C. costata* were counted. The number of eyed-larvae in *C. virginica* was also counted. These larval stages were all expressed as percentages. The dissoconch shell of *M. mercenaria* is difficult to distinguish from its prodissoconch shell. Moreover, the larval foot is difficult to discern in a preserved hard clam specimen. Therefore, only qualitative data were obtained for the final stages of larval development in *M. mercenaria.*

RESULTS

Larval Feeding Experiments

Shell Length

Larvae of *Crassostrea virginica, Cyrtopleura costata* and *Mercenaria mercenaria* fed different diets showed significantly different mean shell lengths as indicated by one-way ANOVA. The mean shell lengths on the final sampling dates and their groupings as determined by SNK tests are summarized in Table 1.

Among diets consisting of individual species, *Isochrysis* aff. *galbana, Ellipsoidon* sp. and *Chaetoceros muelleri* supported larval growth, while *Nannochloris* sp. did not support growth in any of the three bivalve species. *Ellipsoidon* sp. was equally as good as *I.* aff. *galbana* in supporting larval growth in *Mercenaria mercenaria, Crassostrea virginica* but not *Cyrtopleura costata* (Table 1).

Diets composed of more than one species can enhance growth (Table 1). For example, *Mercenaria mercenaria* fed on diets consisting of the three species *Chaetoceros muelleri, Ellipsoidon* sp. and *Isochrysis* aff. *galbana* or *Ellipsoidon* sp., *I.* aff. *galbana* and *Nannochloris* sp. or the diet consisting of all four species *C. muelleri, Ellipsoidon* sp., *I.* aff. *galbana* and *Nannochloris* sp., exhibited more rapid growth than on any of the component species of unialgal diets. Of the mixed diets, the three-species diet consisting of *C. muelleri, Ellipsoidon* sp. and *I.* aff. *galbana* was the most advantageous. It was as good as or better than the best unialgal diets in supporting growth in all three bivalve species (Table 1).

Survival

Data on survival of non-fed larvae were variable. Whereas all fed larvae showed some survival until the final sampling dates, this was not the case for all non-fed larvae. The non-fed larvae of *Mercenaria mercenaria* in both experiments MM1 and MM2, *Crassostrea virginica* in experiment CV1 and *Cyrtopleura costata* in experiment CC1 survived until the final sampling date. In contrast, non-fed larvae of *C. virginica* in experiment CV2 and *C. costata* in experiment CC2 were all dead on the 13th or 9th day, respectively, after fertilization.

Whereas the analysis of variance of the survival percentages of bivalve larvae indicated significant differences among treatments in all experiments, the Student-Newman-Keuls test failed to differentiate among treatments in experiment CV1 (Table 1). Moreover, survival of larvae did not seem to correlate with either unialgal or mixed diets offered.

Metamorphosis

Crassostrea virginica larvae fed unialgal diets of *Chaetoceros muelleri, Isochrysis* aff. *galbana* or *Ellipsoidon* sp. developed into eyed-larvae, but not those fed with *Nannochloris* sp. nor the non-fed larvae. *Ellipsoidon* sp. and *I.* aff. *galbana* supported metamorphosis in *C. virginica* of 1.3% and 5.0%, respectively. Doubling the amount of *I.* aff. *galbana* offered to *C. virginica* quintupled the number of metamorphosed larvae. *Ellipsoidon* sp. was the only unialgal diet that allowed metamorphosis to occur in *Cyrtopleura costata* larvae.

Of the mixed diets composed of three or four species, the three-species diet composed of *C. muelleri, Ellipsoidon* sp. and *I.* aff. *galbana* yielded 23.6%, while the four species diet composed of *C. muelleri, Ellipsoidon* sp., *I.* aff. *galbana* and *Nannochloris* sp. yielded 4.9% metamorphosed larvae in *C. virginica.* The high percentage of metamorphosed larvae in *C. virginica* fed on the three-species diet *C. muelleri, Ellipsoidon* sp. and *I.* aff. *galbana* may be

TABLE 1.

Average shell lengths (μm) and arcsine transformed survival percentages (p′) of bivalve larvae fed on different algal diets on subsequent times (days) after fertilization. p′ = arcsin $\sqrt{p}$, where p = proportion of surviving larvae. Averages with superscripts of similar letters are not significantly different according to Student-Newman-Keuls test (α = 0.05). Feeding ration was based initially on 25,000 cells/larva, unless otherwise indicated. md = missing data, nd = no data, n = number of replicates (beakers).

Diet	*Mercenaria mercenaria* Length	n	Survival	n	*Crassostrea virginica* Length	n	Survival	n	*Cyrtopleura costata* Length	n	Survival	n
	Experiment MM1 (Day 9)				*Experiment CV1 (Day 17)*				*Experiment CC1 (Day 17)*			
C. muelleri	155^{D}	4	60^{AB}	4	192^{B}	4	46^{A}	4	120^{C}	4	58^{C}	4
Ellipsoidon sp.	160^{CD}	4	79^{A}	4	243^{A}	4	68^{A}	4	205^{AB}	4	56^{CD}	4
I. aff. *galbana*	160^{CD}	4	65^{AB}	4	236^{A}	4	57^{A}	4	232^{A}	4	76^{AB}	4
Nannochloris sp.	99^{E}	4	66^{AB}	4	96^{C}	4	43^{A}	4	80^{D}	4	42^{D}	4
Not Feed	95^{E}	3	70^{AB}	3	120^{C}	4	42^{A}	4	77^{D}	4	54^{CD}	4
C. muelleri *Ellipsoidon* sp. *I.* aff. *galbana*	198^{A}	3	67^{AB}	3	247^{A}	3	68^{A}	4	240^{A}	4	82^{A}	4
C. muelleri *Ellipsoidon* sp. *Nannochloris* sp.	170^{BC}	4	76^{A}	4	229^{A}	4	66^{A}	3	182^{B}	4	67^{BC}	4
C. muelleri *I.* aff. *galbana* *Nannochloris* sp.	149^{D}	3	13^{B}	4	180^{B}	4	50^{A}	4	224^{A}	4	41^{D}	4
Ellipsoidon sp. *I.* aff. *galbana* *Nannochloris* sp.	177^{B}	4	45^{B}	4	189^{B}	4	60^{A}	4	175^{B}	4	69^{BC}	4
C. muelleri *Ellipsoidon* sp. *I.* aff. *galbana* *Nannochloris* sp.	179^{B}	4	47^{AB}	4	238^{A}	3	63^{A}	3	242^{A}	4	60^{C}	4
Error Mean Square	38.9		620.8		413.4		107.2		351.7		54.6	
I. aff. *galbana*	186^{A}	4	61^{AB}	4	198^{AB}	4	70^{A}	4	161^{A}	4	29^{B}	4
Not Feed	117^{D}	4	69^{A}	4	md	md	0^{B}	4	md	md	0^{C}	4
C. muelleri *Ellipsoidon* sp.	147^{C}	4	48^{ABC}	4	163^{BC}	4	70^{A}	4	151^{A}	3	64^{A}	3
C. muelleri *I.* aff. *galbana*	175^{AB}	4	52^{ABC}	4	201^{AB}	4	78^{A}	4	191^{A}	4	58^{AB}	4
C. muelleri *Nannochloris* sp.	133^{C}	4	36^{C}	4	135^{C}	4	60^{A}	4	88^{B}	4	44^{A}	4
Ellipsoidon sp. *I.* aff. *galbana*	185^{A}	4	64^{AB}	4	203^{AB}	4	66^{A}	4	55^{B}	4	34^{A}	4
Ellipsoidon sp. *Nannochloris* sp.	145^{C}	4	53^{ABC}	4	164^{BC}	4	70^{A}	4	73^{B}	4	51^{AB}	4
I. aff. *galbana* *Nannochloris* sp.	166^{B}	4	37^{C}	4	133^{C}	4	74^{A}	4	164^{A}	4	35^{A}	4
I. aff. *galbana* 50,000 cells/ml	195^{A}	4	63^{AB}	4	214^{A}	4	75^{A}	4	157^{A}	4	71^{A}	4
C. muelleri *Ellipsoidon* sp. *I.* aff. *galbana*	183^{A}	3	39^{BC}	4	211^{A}	4	78^{A}	4	nd	nd	nd	nd
Ellipsoidon sp. 50,000 cells/mL	nd	nd	nd	nd	nd	nd	nd	nd	71^{B}	3	61^{A}	3
Error Mean Square	79.9		112.9		397.6		85.8		491.1		210.8	

misleading because metamorphosis occurred in only one of four replicates. In *C. costata,* the four-species diet composed of *C. muelleri, Ellipsoidon* sp., *I.* aff. *galbana Nannochloris* and the three-species diet composed of *C. muelleri, Ellipsoidon* sp., *I.* aff. *galbana* and *C. muelleri, I.* aff. *galbana, Nannochloris* sp. produced 12.8, 0.5 and 0.4% metamorphosed larvae, respectively.

Metamorphosed larvae of *Mercenaria mercenaria* were difficult to discern in preserved specimens and therefore were not quantified. Nevertheless, qualitative observations

suggested that the three-species diet composed of *C. muelleri, Ellipsoidon* sp., and *I.* aff. *galbana* yield the highest proportion of pediveligers in hard clams.

Microalgal Composition

The gross chemical composition of microalgae and their equivalent calories are shown in Table 2. Among the unialgal diets, *Isochrysis* aff. *galbana* had the highest lipid content, while *Chaetoceros muelleri* had the highest protein and ash contents. *Ellipsoidon* sp. had the highest carbohydrate content, while *Nannochloris* sp. had the lowest protein content (Table 2). Total caloric equivalent per million microalgal cells was greatest in *I.* aff. *galbana* and lowest in *Ellipsoidon* sp. The major food component that contributed to the total caloric content in all four microalgae *C. muelleri, Ellipsoidon* sp., *I.* aff. *galbana* and *Nannochloris* sp. was presumed to be lipid (Table 2).

DISCUSSION

Planktotrophic larvae of *Crassostrea virginica, Cyrtopleura costata* and *Mercenaria mercenaria* rely on external food sources to nourish most of their development because their eggs have limited amounts of yolk. In our experiments, the energy and nutrients associated with *Ellipsoidon* sp. seem sufficient to support larval growth in *M. mercenaria, C. virginica* but not *C. costata*. The total caloric equivalent of *Ellipsoidon* sp. is 9 times lower than that of *I.* aff. *galbana*, yet it was as good as *I.* aff. *galbana* in supporting growth in *M. mercenaria* and *C. virginica*. More-

TABLE 2.

Gross chemical composition of different diets. All values are from 2 replicate cultures except for the carbohydrate contents of *C. muelleri* and *Ellipsoidon* sp., the protein content of *Ellipsoidon* sp. and "other" content of *C. muelleri* (1 replicate). "Other" was presumably lipid and was calculated from the difference between ash free dry weight and the sum of protein and carbohydrate in each diet. Equivalent calories were calculated using the factors 5.65, 4.10, 9.45 cal/mg for protein, carbohydrate and lipid respectively (Crisp, 1971). All values for mixed diets were calculated from unialgal diets.

Diet	Dry Weight ($\mu g/10^6$ cells)	Ash (percentage of dry weight)	Protein (percentage of dry weight)	Carbohydrate (percentage of dry weight)	"Other" (percentage of dry weight)	Protein (10^6 cells)	Carbohydrate (10^6 cells)	"Other" (10^6 cells)	Total (10^6 cells)
C. muelleri	118	19.76	32.20	20.33	27.71	0.21	0.10	0.31	0.62
Ellipsoidon sp.	17	12.50	29.40	35.30	22.90	0.03	0.02	0.04	0.09
I. aff. *galbana*	118	16.53	11.40	9.80	62.20	0.08	0.05	0.69	0.82
Nannochloris sp.	59	10.14	7.60	22.50	59.70	0.03	0.05	0.33	0.41
C. muelleri *Ellipsoidon* sp.	68	16.13	30.80	27.82	25.31	0.12	0.06	0.18	0.36
C. muelleri *I.* aff. *galbana*	118	18.15	21.80	15.07	44.96	0.15	0.08	0.50	0.72
C. muelleri *Nannochloris* sp.	89	14.95	19.90	21.42	43.71	0.12	0.08	0.32	0.52
Ellipsoidon sp. *I.* aff. *galbana*	68	14.52	20.40	22.55	42.55	0.06	0.04	0.37	0.46
Ellipsoidon sp. *Nannochloris* sp.	38	11.32	18.50	28.90	41.30	0.03	0.04	0.19	0.25
I. aff. *galbana* *Nannochloris* sp.	89	13.34	9.50	16.15	60.95	0.06	0.05	0.51	0.62
C. muelleri *Ellipsoidon* sp. *I.* aff. *galbana*	84	16.26	24.33	21.81	37.60	0.11	0.06	0.35	0.51
C. muelleri *Ellipsoidon* sp. *Nannochloris* sp.	65	14.13	23.07	26.04	36.77	0.09	0.06	0.23	0.37
C. muelleri *I.* aff. *galbana* *Nannochloris* sp.	98	15.48	17.07	17.54	49.87	0.11	0.07	0.44	0.62
Ellipsoidon sp. *I.* aff. *galbana* *Nannochloris* sp.	65	13.06	16.13	22.53	48.27	0.05	0.04	0.35	0.44
C. muelleri *Ellipsoidon* sp. *I.* aff. *galbana* *Nannochloris* sp.	78	14.73	20.15	21.98	43.13	0.09	0.06	0.34	0.49

over, some diet mixtures such as the three-species diet composed of *C. muelleri, Ellipsoidon* sp. and *I.* aff. *galbana* whose total caloric content (Table 2) were also lower than *I.* aff. *galbana,* supported comparable growth (Table 1) suggesting that the total energy demand of the larvae was satisfied at an energy level lower than that contained in *I.* aff. *galbana,* and that some factors in addition to energy content of food were responsible for promoting growth. Davis and Guillard (1958) observed that there was little difference in growth rate of *M. mercenaria* larvae (density = 10–15 larvae/ml) over the range of 50,000 to 400,000 *Isochrysis galbana* Parke cells per ml larval culture water. Riisgard (1988) predicted that the minimum algal concentration for maximum growth in *M. mercenaria* veligers is 40,000 to 60,000 *I. galbana* cells per ml larval culture water (larval density = 11–18 larvae/ml). Increasing the number of *Ellipsoidon* sp. cells offered to *M. mercenaria* from 25,000 to 525,000 per larva (Tan Tiu and Vaughan, unpubl. data) did not increase larval growth suggesting that sufficient energy for growth was obtained from the 25,000 *Ellipsoidon* sp. cells. Doubling the number of *I.* aff. *galbana* cells offered to the larvae of *C. virginica* (experiment CV2) and *C. costata* (experiment CC2) from 25,000 to 50,000 also did not improve their growth. These suggest once again that in our experiments, the initial ration of 25,000 cells per larva was sufficient for larval growth. Note however that the number of *C. virginica* larvae that metamorphosed increased five folds when the number of *I.* aff. *galbana* offered was doubled. This suggests a limiting factor that influences metamorphosis more than the growth of shell length. Doubling the number of *Ellipsoidon* sp. offered to *C. costata* (experiment CC2) did not improve larval growth relative to those fed on *I.* aff. *galbana,* suggesting the relative inferiority of *Ellipsoidon* sp. as feed for *C. costata.*

According to Webb and Chu (1983), the total concentration of cellular protein within algal cells may be important in determining the quality of food. The low amount of protein in *Nannochloris* sp. (Table 2) may be responsible for its poor food quality. The negative effect of *Nannochloris* sp. was diminished when its concentration was decreased to one fourth as in the four-species diet composed of *Chaetoceros muelleri, Ellipsoidon* sp., *Isochrysis* aff. *galbana* and *Nannochloris* sp. Thus, the positive effect possibly due to the balance micronutrients in this four-species diet overcame the negative effect of *Nannochloris* sp., allowing *C. virginica* and *C. costata* larvae fed on such diet to grow and metamorphose. Dupuy (1975) also observed that *Nannochloris oculata* when fed in combination with *Pyramimonas virginica* Pennick, *Pseudoisochrysis paradoxa* (F. Ott, nom. nud.), and *Chrysophaeropsis planktonicus* (Dupuy, nom. prov.) yielded eyed-larvae in *C. virginica* in 11 to 14 days.

The poor food value of some green algae including several species of *Nannochloris* sp. has been attributed to their indigestibility. *Nannochloris* sp., being a chlorophyte, may possess a cell wall; however, since we performed our experiments at 30°C, the cell wall may not have presented a digestibility problem. According to Davis and Calabrese (1964), *Chlorella autotrophica* Shihira and Krauss [=Chlorella 580, (Walne 1970)] was utilized at higher temperatures (up to 30°C) by both *C. virginica* and *M. mercenaria,* although at lower temperature (about 25°C) its cell wall rendered it indigestible, especially to *C. virginica* larvae (Davis and Guillard 1958, Babinchak and Ukeles 1979). Several other factors that influence the nutritional value of food were reviewed by Ukeles (1975) and Webb and Chu (1983). The food value of *Isochrysis* aff. *galbana* has been tested previously with larvae of *C. virginica* (Ewart and Epifanio 1981), *M. mercenaria* (Helm and Laing 1987, Tan Tiu and Vaughan 1988) and *C. costata* (Cresswell and Schilling 1985, Gustafson et al. 1988). Other than Tan Tiu and Vaughan (1988) however, experimental conditions employed by other researchers differed from ours making comparison difficult.

The three-species diet, composed of equal numbers of *Chaetoceros muelleri, Ellipsoidon* sp. and *Isochrysis* aff. *galbana* was the most advantageous mixed diet for larvae of the three bivalve species. This diet supported rapid larval growth (Table 1) in all three bivalve species. The ability of food mixtures to foster growth has been attributed by others to balanced essential nutrients (Bayne 1983, Webb and Chu 1983). Such good food quality in the three-species diet composed of *Chaetoceros muelleri, Ellipsoidon* sp. and *Isochrysis* aff. *galbana* may likewise be due to its more balanced essential nutrients. In this three-species diet, the lipid content is contributed largely by the *I.* aff. *galbana,* while the protein content is furnished mostly by *C. muelleri* (Table 2). *Ellipsoidon* sp. provided the main source of carbohydrate. Additional factors that could influence the food value of these microalgae for these three bivalve species are currently uncertain.

We have yet to find a unialgal diet that can surpass the performance of *Isochrysis* aff. *galbana* in supporting larval growth in *Crassostrea virginica, Cyrtopleura costata* and *Mercenaria mercenaria* under tropical conditions. Nevertheless, we have shown that among the unialgal diets, *Ellipsoidon* sp. can be as good as *I.* aff. *galbana* in supporting larval growth and metamorphosis in *M. mercenaria* and *C. virginica.* The other two microalgae, *Chaetoceros muelleri* and *Nannochloris* sp. can be used as food for these bivalve larvae in some diet mixtures described above. Some of these mixed diets were as good as and/or better than *I.* aff. *galbana* as feeds for bivalve larvae. The performance of the three-species diet composed of *C. muelleri, Ellipsoidon* sp. and *I.* aff. *galbana,* and the unialgal diet of *Ellipsoidon* sp. as feeds for bivalve larvae should be explored further. As these strains all grow at temperature up to 35°C, they may find great utility in hatcheries located in subtropical or tropical countries.

ACKNOWLEDGMENTS

We are grateful to Dr. William Lellis for reviewing the manuscript and helping with statistical analyses. Suggestions of the two anonymous reviewers and Dr. Sandra E. Shumway substantially improved the presentation of this manuscript. The help of DAB staffs in the collection of broodstocks, maintenance of experimental facilities, and discussions is greatly appreciated. We also extend thanks to the Solar Energy Research Institute, Golden Co., for providing reports and microalgal strains. This study was supported by a Harbor Branch Postdoctoral Fellowship to A.T.T. Harbor Branch Oceanographic Institution, Inc. publication contribution number 720.

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