Zoo Biology 25:513–525 (2006)

Research Article

Comparative Growth and Survival of Juvenile Hard Clams, *Mercenaria mercenaria*, Fed Commercially Available Diets

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Hobbyist and researchers often use commercially available phytoplankton concentrates to maintain filter feeding organisms held in their ornamental or experimental tanks. This study investigated the nutritional value of 10 products available commercially for juvenile hard clams, *Mercenaria mercenaria*. Growth and mortality rates in clams fed these products were compared with those found in clams fed fresh cultures of the microalgae *Isochrysis galbana*, which is considered an industry standard for supporting growth of juvenile bivalves. Our results show a clear difference in feed nutritional value between non-living and living commercial diets, and among commercial diets advertised as containing live algae. Overall, results showed that juvenile hard clams fed fresh cultures of *I. galbana* displayed the best growth and lowest mortality rates, followed by those fed the commercial diet DT's Live Marine Phytoplankton. Growth and mortality rates in unfed controls were similar to those found in clams fed commercial non-living algae mixes or diets advertised as containing live algae (Phyto-Feast Live product). Results also showed that the nutritional value of fresh algae (*I. galbana*) cultures is lost rapidly when cultures are maintained at 4°C, suggesting that algae present in some commercial diets may lose their nutritional value during processing or refrigerated storage. The commercial blend, DT's Live Marine Phytoplankton, seems to represent a good substitute to lab grown algae for clams held in ornamental or experimental aquariums. Zoo Biol 25:513–525, 2006.

Keywords: algae; feed; shellfish; aquarium; aquaculture; ornamental

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Received 21 December 2005; Accepted 8 September 2006

DOI 10.1002/zoo.20113
Published online 1 December 2006 in Wiley InterScience (www.interscience.wiley.com).

INTRODUCTION

How to feed marine invertebrate species in an ornamental or experimental aquarium? Aquarists who increasingly use these animals to decorate their reef aquarium or scientists who need to maintain shellfish during experiments frequently ask this question. Most of these animals are species that derive their nutrition from filtering out microscopic food particles suspended in seawater. Choices of available feed for marine invertebrates are becoming more various on the market. Varieties currently include concentrated live phytoplankton cultures, refrigerated or frozen algae pastes, dehydrated algae, preserved liquid room-temperature forms, bacteria, yeast and various types of microcapsules [Coutteau and Sorgeloos, 1992]. Among these diets, refrigerated microalgae concentrates and pastes seem to be the most promising alternatives to fresh phytoplankton cultures, despite the fact that very few experimental studies have been carried out to determine the nutritive value of stored microalgae [Robert and Trintignac, 1997; McCausland et al., 1999; Ponis et al., 2003]. In some experiments, growth patterns and mortalities of shellfish fed with preserved algal cells were comparable to those sustained on live algae [McCausland et al., 1999; Ponis et al., 2003]. However, some algae species have a short shelf life and cannot survive a lengthy maintenance at low temperatures [Ponis et al., 2003]. Hence, such limitations could potentially affect their nutritional value. Most diets available on the market do not furnish details on their specific composition, or the methods under which they were processed. Nevertheless, the choices of algal species, as well as harvesting and preservation techniques, are essential to maintaining algal food value [Heasman et al., 2000].

The objective of this research was to compare the nutritional value among several commercially available diets for juvenile hard clams, *Mercenaria mercenaria*. These diets were also compared with fresh lab grown cultures of *Isochrysis galbana*, which was used as a control. Refrigerated lab grown mono-specific algal cultures were used in our trials in an attempt to evaluate the effect of refrigerated storage on algae nutritional value. These algae were also microscopically examined to document any alteration to the integrity of algal cells during refrigerated storage. Clam growth and survival were monitored on a weekly basis.

MATERIALS AND METHODS

Clams

Juvenile (1–2 mm) hard clams, *Mercenaria mercenaria*, were obtained from Bay Shellfish Company (Terra Ceia, FL). All experiments were carried out at Flax Pond Marine Laboratory (Stony Brook University, NY). Clams were held in 3-L tanks (500 clams per tank) containing 1L aerated, filtered (2 μm cartridges) and UV sterilized seawater. All experiments were conducted at room temperature ($22 \pm 1^{\circ}C$), and salinity was maintained at 29 ± 1 ppt. Seawater in each tank was replaced every other day to maintain water quality.

Experimental Diets

Experiment 1

This experiment was designed to compare the nutritional value of preserved non-living phytoplankton. Seven commercial diets were evaluated with respect to

their contents (identification of major algal constituents) and their ability to support clam growth and survival (Table 1). They included PhytoPlex, PhytoMax, ChromaPlex and ChromaMax (Kent Marine, Franklin, WI), PhytoPlan and Marine Snow (Two Little Fishies, Coconut Grove, FL), and BioPlankton (LiquidLife USA, Gardena, CA). Because earlier investigations had suggested that viable phytoplankton cells provide a better nutritional resource than non-viable cells [Robert and Trintignac, 1997], an eighth product (DT's Live Marine Phytoplankton; DT's Plankton Farm, Sycamore, IL), advertised as containing live phytoplankton cells, was also used during these comparative assays. Each commercial blend was preserved according to manufacturer's recommendations. The blends were examined to determine their algae species composition. Clams fed these products were compared with a negative (unfed) control group and to clams fed with fresh *Isochrysis galbana* (positive control), which is considered a standard for supporting growth of juvenile bivalves [Walne, 1970] (Table 1). An initial inoculum of *I. galbana* (strain CCMP 1323) was provided by Dr. Gary Wikfors (NOAA, National Marine Fisheries Service, Milford, CT). This strain was propagated in 2 × 5-L flasks on F/2 medium [Guillard and Ryther, 1962], under continuous lighting and injected with air bubbling in our laboratory (14°C, 31 ± 1 ppt, pH between 7.8–8.5).

Experiment 2

This experiment compared the nutritional value among commercial diets advertised as live phytoplankton and investigated the effect of refrigerated preservation on the nutritional quality of selected phytoplankton species. Two commercial diets were evaluated with respect to their contents (identification of major algal constituents) and to their ability to support clam growth and survival (Table 2): Phyto-Feast Live Marine Microalgae (Reed Mariculture, Campbell, CA) and DT's Live Marine Phytoplankton (DT's Plankton Farm). A third commercial product (Phyto-Feast Coral and Clam, Reed Mariculture), advertised as having the "same nutritional profile as live algae," was used during these comparative assays. Each commercial blend was examined to determine its algal composition. The effect of refrigeration on the nutritional value of phytoplankton was tested on lab grown cultures of *I. galbana* (see above), *Pavlova* sp. (strain CCMP 459), and *Tetraselmis striata* (PLAT-P), which were also provided by Dr. Gary Wikfors (NOAA, NMFS). Each strain was grown in 2 × 20 L carboys on F/2 medium, under culture conditions described above. Exponentially growing cultures were transferred to the refrigerator (4°C) and used in the feeding experiment that started on the following day. Clams fed these diets were compared with a negative (unfed) control group and to clams fed with fresh *I. galbana* (positive control) for a period of 10 weeks. On a weekly basis, live blends and preserved algae were observed under a Nikon inverted microscope to evaluate the integrity of algal cells. Digital photographs were taken to document their state.

Feeding Protocol

Due to the wide variety of diets being examined, the most consistent way to establish comparable feeding rates is on a dry weight basis. The dry weight content (g 100 mL^{-1}) of all diets was determined at the start of each experiment. This was done by filtering up to 100 ml of each diet through a tared glass fiber filter and drying at 60°C until weight stabilization. Three samples of each diet were filtered and

TABLE 1. Experimental diets and rations used in Experiment 1[a]

Product	Producer	Species (manufacturer's data)	Dry weight (g/100 ml, mean ± SD)	Volume (ml) or weight (g) added to each tank
Phytoplex	Kent Marine	*Nannochloropsis* sp. *Tetraselmis* sp. *Isochrysis* sp.	0.21 ± 0.01	6.64
Phytomax	Kent Marine	*Nannochloropsis* sp. *Tetraselmis* sp. *Isochrysis* sp.	13.61 ± 0.70	0.10
Chromaplex	Kent Marine	N.C.	0.32 ± 0.00	4.37
Chromamax	Kent Marine	N.C.	15.67 ± 0.15	0.09
PhytoPlan	Two Little Fishies	*Arthrospira* sp. *Haematococcus* sp. *Schizochytrium* sp.	60.64 ± 4.14 g/100 g (Commercialized as a powder)	0.02
Marine Snow	Two Little Fishies	*Nannochloropsis* sp. *Tetraselmis* sp. **or** *Thalassiosira* sp. *Isochrysis* sp. *Spirulina* sp. *Schizochitrium* sp. **and** Seaweed meal, Zooplankton, Citric Acid	0.31 ± 0.03	4.50
BioPlankton	LiquidLife U.S.A.	*Nannochloropsis* sp. *Tetraselmis* sp. *Isochrysis* sp.	24.97 ± 2.86	0.06
DT Live Marine Phytoplankton	DT's Plankton Farm	*Nannochloropsis oculata* *Nannochloropsis salina* *Chlorella* sp.	0.40 ± 0.02	3.49
Isochryis galbana	Fresh lab grown culture	*Isochryis galbana* (CCMP 1323)	NA	$120.10^6/N$ N = cell number.ml^{-1} in the culture

[a]Species composition is listed according to each manufacturer's description. The last column represents the volume (weight for Phytoplan) added daily to each tank containing 500 clams (calculation based on diets dry weight and clam dry weight which was equal to 0.093 g/500 clams; daily food ration representing 15% of animal weight). For *I. galbana*, the relationship between dry weight and cell numbers was determined before the beginning of the experiment and the volume of the daily food ration was calculated as $V = 120.10^6/N$ (N = cell number/ml; see text for more details). NC, non-communicated.

TABLE 2. Experimental diets and rations used in Experiment 2[a]

Product	Producer	Species (manufacturer's data)	Dry weight (g/100 ml)	Volume (ml) added to each tank
Phyto-Feast LIVE Marine Microalgae	Reed Mariculture	*Amphora* sp. *Isochryis* sp. *Nannochloropsis* sp *Pavlova* sp. *Tetraselmis* sp. *Thalassiosira weissflogii*	2.71 ± 0.37	0.61
Phyto-Feast Coral & Clam Diet	Reed Mariculture	*Amphora* sp. *Isochryis* sp. *Nannochloropsis* sp. *Pavlova* sp. *Tetraselmis* sp. *Thalassiosira weissflogii*	5.93 ± 1.23	0.28
DT Live Marine Phytoplankton	DT's Plankton Farm	*Chlorella* sp. *Nannochloropsis oculata* *Nannochloropsis salina*	0.47 ± 0.08	3.56
Isochryis galbana	Refrigerated lab grown culture	*Isochryis galbana* CCMP 1323	0.05 ± 0.01	32.80
Pavlova sp.	Refrigerated lab grown culture	*Pavlova* sp. CCMP 459	0.05 ± 0.01	33.30
Tetraselmis striata	Refrigerated lab grown culture	*Tetraselmis striata (Plat P)*	0.06 ± 0.01	27.00
Isochryis galbana	Fresh lab grown culture	*Isochryis galbana* CCMP 1323	NA	$120.10^6/N$ N = cell number.ml^{-1} in the culture

[a]Species composition is listed according to each manufacturer's description. The last column represents the volume added daily to each tank containing 500 clams (calculation based on diets dry weight and clam dry weight which was equal to 0.109 g/500 clams; daily food ration represents 15% of animal weight). For fresh culture of *I. galbana*, the relationship between dry weight and cell numbers was determined before the beginning of the experiment and the volume of the daily food ration was calculated as $V = 120.10^6/N$ (N = cell number/ml, see text for more details). NA, non-applicable.

weighed, and the average of the three was calculated for feeding rate determinations. The dry weight of Phytoplan, a product commercialized as a powder, was determined by incubating three replicates of 10 g each at 60°C until weight stabilization. The mean dry tissue weight of clams was determined by dissolving shell material (DeCal Solution; Fisher-Scientific, Pittsburgh, PA) from three 50-clam sub-samples, rinsing tissues with distilled water, and then drying at 60°C until weight stabilization. All diets were provided to clams at the rate of 15% dry weight/day [Epifanio and Ewart, 1977]. The biomass (ultimately the dry weight) in *I. galbana*

cultures changes with culture stage-of-growth and algae cell count. The relationship between cell count and dry weight of cultured *I. galbana* was established at the beginning of each experiment and culture cell counts were determined before each feeding to ensure experimental conditions similar to those for commercial diets (15% dry weight/day). Each diet was provided to three replicate groups of 500 juvenile clams held in 3-L tanks containing 1 L of aerated, filtered seawater. Clams were maintained as described above (31 ± 1 ppt; $22 \pm 1^{\circ}$C, water in each tank was replaced every other day).

Clam Growth and Mortality

Clam growth was evaluated weekly. A representative sub-sample of clams (~50 clams) was removed from each tank and placed under a Nikon stereo-dissecting microscope. Digital images of each sample were captured. The shell area (cm^2) of each of the 50 clams was measured using image analysis software (ImageJ, National Institute of Health). Clam survival was determined by counting and removing all dead clams on a weekly basis. Experiments were terminated after ten weeks by sampling all remaining clams for size measurements and mortality counts. Growth and mortality were statistically compared as a function of diet, using one-way analysis of variance and Fisher's least significant difference (LSD). Mortality data were arcsin-transformed before statistical analysis. Results were considered significant if $P < 0.05$.

RESULTS

Experiment 1

Composition and ration of experimental diets

The composition of experimental diets is presented in Table 1. These data were compiled from information provided by each manufacturer, because the identification of specific algae was not possible in most of these products, probably due to cellular damage inflicted during product processing. Several products contained cell debris or cells that were totally empty of their cytoplasmic contents. Although the producer of Marine Snow (MS) declares up to five different microalgae species, their presence was not detected in this product and only an insignificant amount of *Tetraselmis* sp. (8–10 μm in diameter) cells was observed. On the other hand, cells present in the viable DT's Live Marine Phytoplankton (DT) presented intact walls and chloroplasts. The commercial diets were extremely heterogeneous with regard to dry weight, with values ranging from 0.21 ± 0.01 to 24.97 ± 2.86 g/100 ml of product (Table 1). Consequently, the daily rations, which represent 15% of 0.093 g (the dry weight of 500 clams), ranged from 0.06–6.64 ml per tank (Table 1). Phytoplan was added at 0.023 g per tank. For *I. galbana*, cell counts in the semi-continuous cultures were determined daily and the volume used to feed the 500 clams was calculated using the following equation: $V = 120.10^6/N$, where N is the number of cells per ml in the semi-continuous culture. This volume was determined before the beginning of the feeding trials by establishing the relationship between cell count and dry cell weight in exponentially growing semi-continuous cultures of *I. galbana*.

Clam Growth and Mortality

At the start of the experiment, the mean shell area was equal to $0.04 \pm 0.001\ cm^2$ (mean $\pm$ SEM) (Fig. 1). Four weeks after beginning the experiment, clams fed with DT's live marine phytoplankton ($0.055 \pm 0.001\ cm^2$) and lab grown *I. galbana* ($0.053 \pm 0.001\ cm^2$) displayed significantly larger shells than animals fed with other diets ($P < 0.05$, ANOVA followed by a Fisher's LSD). Shell area ranged between $0.046 \pm 0.001\ cm^2$ in clams fed with Phytoplex and $0.043 \pm 0.001\ cm^2$ in those fed with Phytoplan (negative control = $0.045 \pm 0.001\ cm^2$). After 8 weeks, this tendency was confirmed and it persisted until the end of the experiment (10 weeks, $P < 0.05$). The leader group represented clams fed with *I. galbana* ($0.059 \pm 0.001\ cm^2$) and DT's live marine phytoplankton ($0.058 \pm 0.001\ cm^2$). The growth obtained in the other groups ranged between $0.039 \pm 0.001\ cm^2$ (negative control) and $0.044 \pm 0.001\ cm^2$ (Chromaplex, Phytoplex).

The highest cumulative mortality was measured in clams fed with Bioplankton, where it reached $11.1 \pm 3.2\%$ at the end of the experiment (Fig. 2). In the other tanks, the mortality was significantly lower ($P < 0.05$, ANOVA) and varied between $2.8 \pm 0.3\%$ for the unfed control and $4.0 \pm 0.5\%$ for clams fed with *I. galbana*.

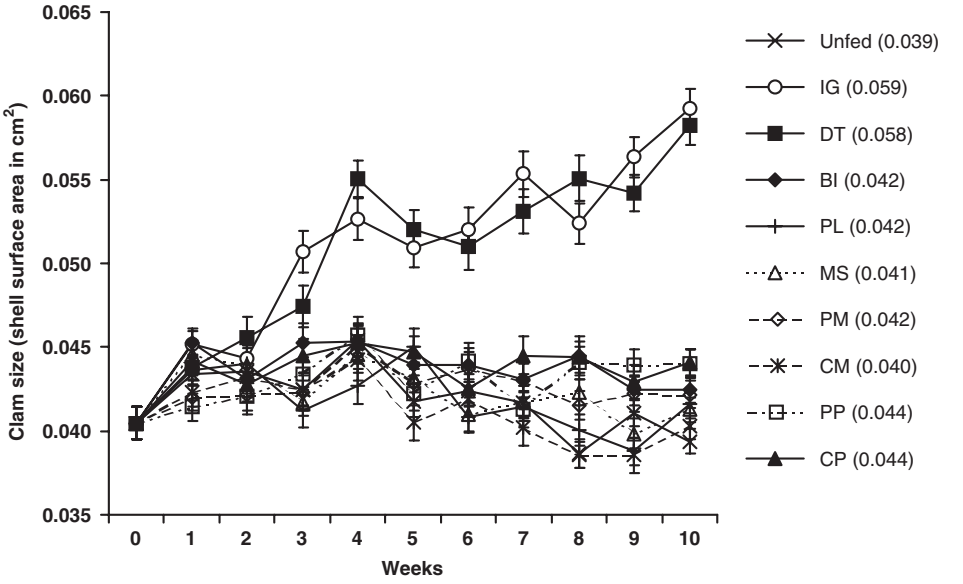

Fig. 1. Growth of clams fed non-living commercial diets (shell area in cm^2, mean $\pm$ SEM). The diet DT's Live Marine Phytoplankton (DT), advertised as containing live phytoplankton cells, was also used during these comparative assays. Unfed clams and those fed lab grown *Isochrysis galbana* (IG) cultures were used as negative and positive controls, respectively. BI, Bioplankton; PL, Phytoplan; MS, Marine Snow; PM, Phytomax; CM, Chromamax; PP, Phytoplex; CP, Chromaplex. IG and DT induced a significantly higher growth rate (ANOVA, $P < 0.05$) compared with all other diets starting 3 and 4 weeks after the beginning of the experiment, respectively.

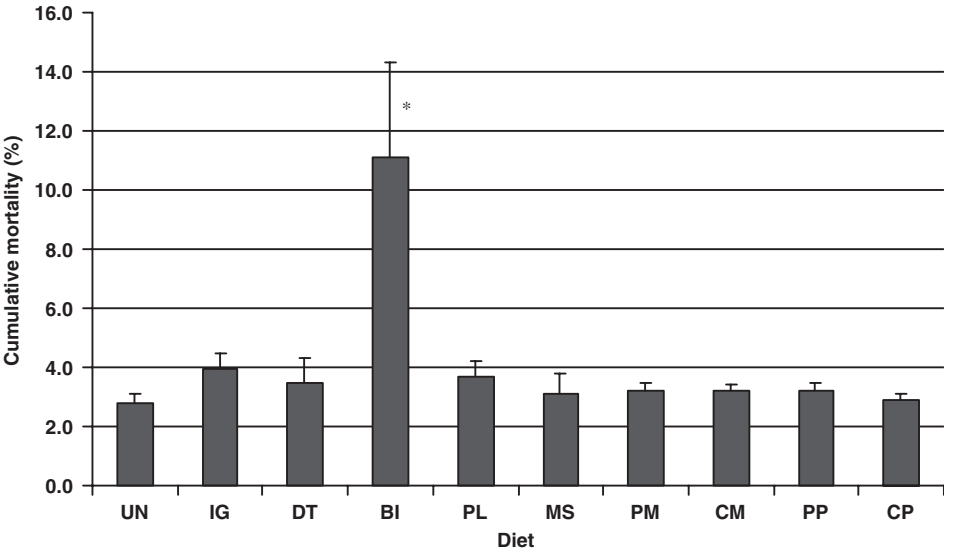

Fig. 2. Cumulative mortality in clams fed non-living commercial diets. See Figure 1 legend for more details. *Significantly higher mortality than all other batches (ANOVA, $P<0.05$).

Experiment 2

Diet composition and ration of experimental diets

Phyto-feast Coral & Clam Diet (CC) and Phyto-feast Live Marine Microalgae (PF) seem to have the same algae composition with CC being more concentrated than PF (Table 2). Almost all the algal cells in these two blends presented intact wall and chloroplasts. Cells observed in the viable DT's Live Marine Phytoplankton (DT) also presented intact wall and chloroplasts. The dry weight of these commercial diets ranged from 0.47 ± 0.08 (DT) to 5.93 ± 1.23 g (CC) per 100 ml of product (Table 2), and the daily rations, which represent 15% of 0.109 (the dry weight of 500 clams) ranged from 0.28 (CC) to 3.56 (DT) ml per tank. For the refrigerated lab grown culture, *I. galbana*, *Pavlova* sp., and *T. striata*, the daily volume added was 32.8, 33.3 and 27.0 ml per tank, respectively. The volume of fresh *I. galbana* culture added to each tank was determined daily, as described in Experiment 1.

Diet preservation

After 10 weeks of refrigerated storage, >90% of the cells present in the DT product showed intact walls and chloroplasts and only very few empty cells (ghosts) or cell debris. In both CC and PF, no obvious difference concerning cell integrity was noticed throughout the 10 weeks of refrigerated storage. At the beginning of the experiment, all refrigerated *I. galbana* cells presented intact walls and chloroplasts. Damaged and aggregated empty cells were noticed after 1 week of refrigerated storage. After 4 weeks, approximately 75% cells appeared intact. After 10 weeks, only 10–20% of the cells presented intact walls. Similarly, aggregates of damaged and empty cells appeared in *Pavlova* sp. cultures after 1 week of refrigerated storage. After 4 weeks, close to 50% cells appeared intact. After 10 weeks, only

about 1–2% of the cells presented intact chloroplasts and cell walls. Damaged and empty *T. striata* cells forming aggregates represented about 20% after 7 weeks of refrigerated storage. After 10 weeks, between 60–70% of the cells remained intact.

Clam growth and mortality

At the beginning of the experiment, the mean shell area was equal to $0.050 \pm 0.002\,cm^2$ (mean $\pm$ SEM) (Fig. 3). Two weeks later, clams fed with fresh *I. galbana* displayed significantly larger shells ($0.061 \pm 0.001\,cm^2$) than animals fed with other diets ($P < 0.05$, ANOVA followed by a Fisher's LSD). Shell areas ranged from $0.054 \pm 0.001\,cm^2$ in clams fed DT's live marine phytoplankton to $0.051 \pm 0.001\,cm^2$ in clams fed refrigerated *Pavlova* sp., Phyto-Feast Live and in the unfed control. After 5 weeks, clams sustained on fresh *I. galbana* ($0.094 \pm 0.002\,cm^2$) and DT's live marine phytoplankton ($0.067 \pm 0.001\,cm^2$) were significantly larger ($P < 0.05$, ANOVA) than those fed with the other products or the negative controls.

At the end of the experiment, unfed controls displayed the highest cumulative mortality at $74.1 \pm 3.2\%$ (Fig. 4), followed by clams fed with refrigerated *T. striata* ($58.7 \pm 1.8\%$). In an intermediate group comprised of clams fed refrigerated *I. galbana* and *Pavlova* sp., cumulative mortality was $48.4 \pm 1.6\%$ and $48.3 \pm 3.2\%$, respectively. Clams fed with Phyto-Feast Coral and Clam and Phyto-Feast Live showed a cumulative mortality of $36.6 \pm 2.7\%$ and $37.9 \pm 0.4\%$, respectively. The lowest mortality was found in clams fed fresh *I. galbana* ($22.9 \pm 0.6\%$) and DT's live marine phytoplankton ($25.3 \pm 2.5\%$).

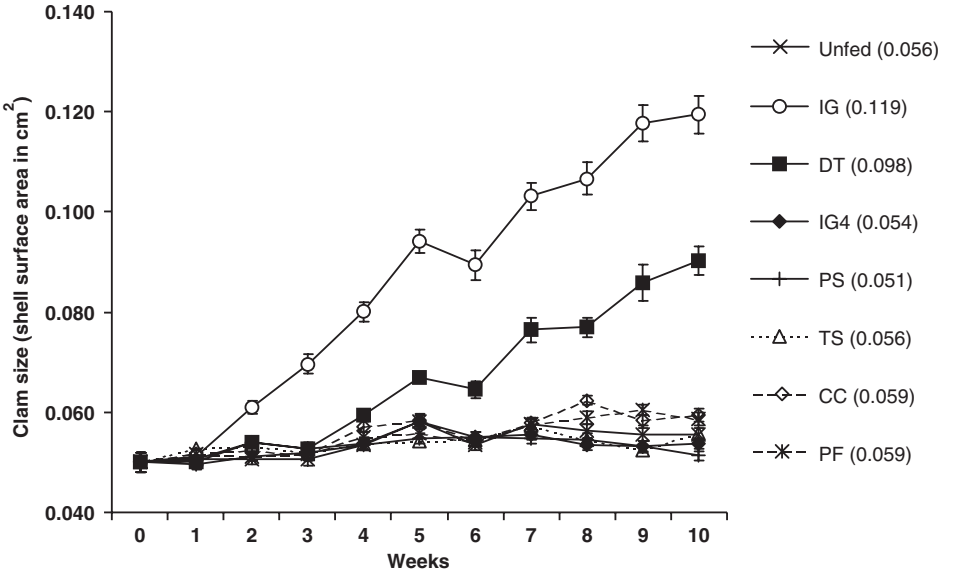

Fig. 3. Growth of clams fed commercial diets or lab grown microalgae (shell area in cm^2, mean $\pm$ SEM). IG, fresh lab grown *Isochrysis galbana*; DT, DT's Live Marine Phytoplankton; IG4, refrigerated cultures of *I. galbana*; PS, refrigerated cultures of *Pavlova* sp.; TS, refrigerated cultures of *Tetraselmis striata*; CC, Phyto-Feast Coral and Clam; PF, Phyto-Feast Live Marine Microalgae. IG and DT induced a significantly higher growth rate (ANOVA, $P < 0.05$) compared with all other diets starting 2 and 5 weeks after the beginning of the experiment, respectively.

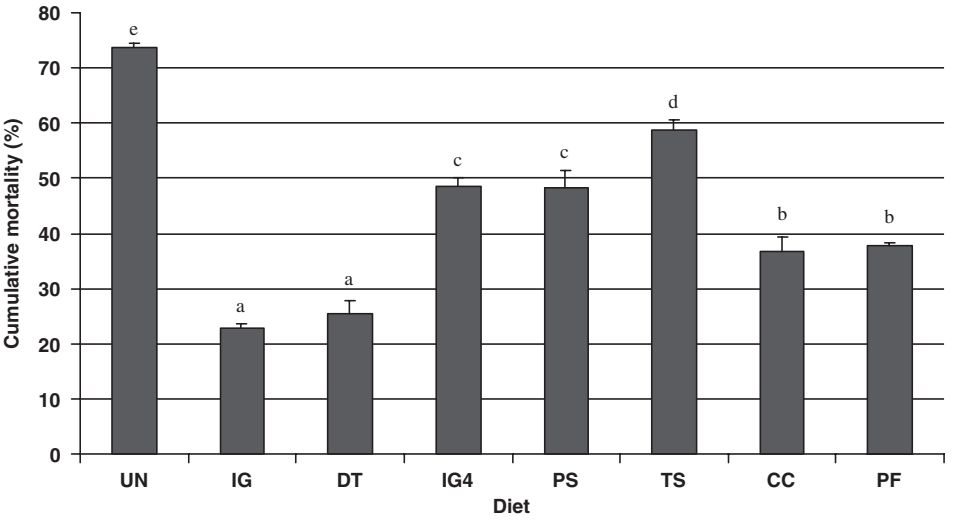

Fig. 4. Cumulative mortality in clams fed commercial diets or lab grown microalgae. See Figure 3 legend for more details. **a–e:** Differences among clams fed different diets (ANOVA, $P<0.05$).

DISCUSSION

Results of these experiments showed that live cultured *Isochrysis galbana* generates higher growth rates in juvenile hard clams, *Mercenaria mercenaria*, than any tested commercial diet. Among the investigated commercial diets, DT's Live Marine Phytoplankton induced the best growth and was comparable to growth obtained in clams fed fresh *I. galbana*. This observation was most apparent in the first experiment. Non-living commercial diets and Phyto-Feast Live products proved inadequate in supporting hard clam growth. Similarly, feeding clams with refrigerated mono-specific lab grown cultures does not support clam growth. Our results show a clear difference in the nutritional value between non-living and living commercial diets, and among commercial live diets.

A difference found in clams fed live diets over those fed non-living diets is not surprising because prior studies had shown that microalgal pastes and dried microalgae yield lower growth rates than fresh microalgal diets in bivalves [Laing and Millican, 1991; Robert and Trintignac, 1997]. Prior studies had also shown that algae species differ among themselves in terms of nutritional value to bivalve filter feeders [Walne, 1970; Chretiennot-Dinet et al., 1986]. The fact that DT's Live Marine Phytoplankton was more efficient than Phyto-Feast products for the growth of juvenile clams may have partial basis on the specific composition of each of these commercial products. In addition to the intrinsic nutritional value of each algae species, there is evidence that the processing methods used to collect, concentrate and preserve algae species affect their ultimate nutritional value. For instance, the nutritional value in refrigerated *I. galbana* cultures was quickly lost, as a significant difference in growth appeared within 2 weeks between animals fed this diet and those fed with fresh cultures of the same algae species. In fact, refrigeration of *I. galbana* (and *Pavlova* sp.) cultures seemed to quickly induce cell degradation, as evidenced

by cell debris and ghost cells within the first week of maintenance at 4°C. Earlier studies had showed that some microalgae species have a short shelf life. For instance, juvenile oysters *Crassostrea gigas* fed concentrated blends (*Pavlova lutheri*, *I. galbana*, and *Chaetoceros calcitrans*) and stored at 1°C for a 4-week period showed significantly lower growth compared with the corresponding fresh microalgae [Ponis et al., 2003]. The value of an energetic supply from a living microalgal cell vs. that of a damaged/dead one could be at the origin of some of the differences observed in the present study. Ponis et al. [2003] reported that whereas some species (*P. lutheri*, for example) did not exhibit significant changes in gross composition (protein, lipid, and carbohydrate) over a 4-week storage at 1°C, other concentrates (*I. galbana*) showed a dramatic decrease in protein and carbohydrate content during the first week of storage. In the same way, the organic matter composition of concentrated *C. calcitrans* cells decreased significantly after 3 weeks storage under the same conditions.

Although *Tetraselmis striata* cells appeared in a relatively good state (cell wall and chloroplast integrity) after 10 weeks' storage, clams fed with this diet do not present significant growth compared with negative controls. Refrigeration does not seem to alter food value of this genus. Indeed, Montaini et al. [1995] have reported that storage at 4°C does not modify viability and biochemical composition of *Tetraselmis suecica*, in particular the fatty acids that play an important role in the quality of the species as food in aquaculture. Previous works have shown contradictory results with regard to the growth of bivalves fed with *T. suecica* [Walne, 1970; Langdon and Waldock, 1981; Laing and Millican, 1986; O'Connor et al., 1992; Albentosa et al., 1993]. It has been suggested that this contradiction might be caused by the particular bivalve species, its developmental stage, or the algae clone used in each experiment [Robert et al., 2001]. Overall, this genus, fresh or preserved, seems to be valuable as a food for filter feeders as long as it is mixed with other algal species and not used as mono-specific feed [Robert et al., 2001].

In addition to the effect of refrigeration and preservation on the biochemical composition (and consequently nutritional values) of algae cells, differences in the growth of clams fed different diets may also stem from the ability of bivalve filter feeders to selectively ingest food particles, rejecting unwanted ones as pseudofeces [Shumway et al., 1985]. For instance, food particles present in diets that did not induce any growth in clams (non-viable commercial diets, Phyto-Feast products and refrigerated lab grown cultures), because of alterations in their physical or biochemical characteristics during processing and preservation, may have been largely rejected as pseudofeces. Ward et al. [1998] showed that both oysters *C. gigas* and mussels *Mytilus trossulus* are able to sort and ingest live microalgal cells of *Rhodomonas lens* and selectively reject in their pseudofeces dried and crushed particles (3–20 μm) of the smooth cord grass *Spartina alterniflora*. Such rejection of low quality particles in favor of higher quality microalgal cells has been shown by several investigators [Newell et al., 1989; Newell and Shumway, 1993; Pastoureaud et al., 1996]. The processing of some of the tested commercial diets (all non-viable diets and Phyto-Feast products) and refrigerated lab cultures may have caused physical alterations to the cells, leading to their selective rejection as pseudofeces.

Clam mortality was relatively low in the first experiment, particularly in unfed clams. This result indicates the ability of juvenile clams to survive a long period

of starvation. Surprisingly, mortality rates were significantly higher in clams fed with Bioplankton (BI), as compared with all other diets. These results suggest a deleterious effect of this commercial product on juvenile clams. Mortality rates in the second experiment were significantly higher than those encountered during the first experiment. It is noteworthy to mention that both experiments used the same experimental setup and that the only variables were clams (not the same clam spawn) and diets. However, the fact that mortality was also higher in the control clams suggest that the second experiment may have used clams of poorer general condition than those used in the first trial. The lowest mortality rates measured during the second experiment were obtained in clams fed with fresh *I. galbana* and DT's Live Marine Phytoplankton, results in agreement with growth data, suggesting that these mortality rates provide a good estimate of the nutritional value of each diet feed.

CONCLUSIONS

1. Juvenile hard clams fed fresh cultures of *Isochrysis galbana* displayed the best growth and lowest mortality rates, followed by those fed with DT's Live Marine Phytoplankton.
2. Growth and mortalities rates in unfed controls were similar to those found in clams fed with refrigerated mono-specific cultures (*Isochrysis galbana*, *Pavlova* sp., and *Tetraselmis striata*), the other commercial non-living algae mixes, and both Phyto-Feast blends.
3. The commercial blend, DT's Live Marine Phytoplankton, seems to represent a good substitute of lab grown algae for hard clams held in ornamental or experimental aquariums.

ACKNOWLEDGMENTS

We are grateful to Bay Shellfish Company (Terra Ceia, Florida) for providing experimental clams, and to Mrs. D. Tsang for reviewing an early draft of this manuscript.

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