Use of Spray-dried *Schizochytrium sp.* as a Partial Algal Replacement for Juvenile Bivalves.

Phil Boeing

Pacific Seafarms International S.A. de C.V.
Cerro Escondido #122
Mazatlan, Sinaloa, Mexico.

ABSTRACT

Two commercially important species of juvenile bivalves (*Tapes semidecussata* and *Crassostrea gigas*) were fed a spray-dried preparation of heterotrophically grown algae, *Schizochytrium sp.* in 20 L recirculating downwell systems. All systems were fed a weekly ration equal to 50% of their live weight as dry weight calculation for live algae or percentage *Schizochytrium sp.* substitute.

Weekly growth rates of juveniles as live wet weight increase were measured to determine the effects of the different diets. Two experiments were performed in duplicate, the first using *Tetraselmis suecica*, a live algae control of moderate nutritional value, and the second using an equal mixture of *Chaetocerous sp*. and *Tetraselmis suecica,* a live algae control of high nutritional value. In the first experiment, 40% and 80% substitution for live algae *Tetraselmis suecica* were compared to controls fed 100% live *Tetraselmis suecica*. Significantly higher growth over the control was obtained with 40% substitution in *Crassostrea gigas* and 40% and 80% substitution in *Tapes semidecussata*. For the second experiment, 40% and 80% substitution for equal portions of live algae *Chaetocerous sp*. and *T. suecica* mixture were compared to controls fed 100% live algae mixture. Significantly lower growth rates were found for *Crassostrea gigas* at both 40% and 80% substitution. However no significant growth difference was found for *Tapes semidecussata* at 40% substitution, in contrast to a significantly lower growth over the control at 80% substitution. These results suggest that the routine use of *Schizochytrium sp*. as a partial replacement of live algae in bivalve culture to be economically viable, depending upon the unit production cost of the live algae for any given nursery facility.

Nursery culture of bivalve molluscs is a useful step for improving required growout time and survival in temperate climates (Anderson and Chew 1982; Bayes 1991). Algal systems for feeding bivalve nurseries represent a major production cost factor for shellfish enterprises (Coutteau and Sorgeloos 1992; Laing and Millican 1992; Meyers and Boisvert 1990; Walsh 1987).

Artificial and replacement diets for bivalve molluscs have been developed and extensively evaluated (Albentosa et al. 1989; Boeing 1986; 1992; Jones et al. 1974; 1993; Langdon et al. 1985; Langdon and Siegfried 1984; Langdon and Waldock 1981; Urban and Langdon 1984). Apparently none of these diets has shown sufficient cost-effectiveness to be commercially practical. The possibility of using heterotrophically grown spray-dried algae as a partial or total replacement for live algae has also seen considerable research, although with only a few algae species (Curtolo et al. 1993; Helm and Hancock 1990; Hidu and Ukeles 1962; Laing et al. 1990; Laing and Millican 1991; 1992; Laing and Verdugo 1991). Despite reported early sucesses with hetertrophically grown spray-dried algae species such as *Tetraselmis suecica* and *Cyclotella cryptica*, several investigators have pointed out the need for more nutritionally valuable species to be made available for the bivalve industry (Laing and Verdugo 1991; Jones et al. 1993). A recently commercialized heterotrophically grown spray-dried marine algae, *Schizochytrium sp.* (ALGAMAC-2000, Aquafauna Bio-Marine, Los Angeles, CA.) has shown very good acceptability and performance against controls when used at up to 70% algal replacement for penaeid shrimp (Boeing 1996; Rosenberry 1996). This product has nutritional qualities which would seem to make it an excellent candidate for algal substitution in other aquatic species such as bivalves and fish.

The present paper describes some preliminary results of substituting Schizochytrium sp. for live algae *Tetraselmis suecica* and *Chaetoceros sp*. in the nursery culture of juvenile *Crassostrea gigas* and *Tapes semidecussata*.

MATERIALS AND METHODS

Lots of 2-4 mm *Tapes semidecussata* and 4-6 mm *Crassostrea gigas* from the same cohort were used in the experiments. Two independent trials for both species were set up and carried out in duplicate. For both experiments animals were dried on absorbent paper and lots of 1.5 g and 2.5 g were weighed out for *Tapes semidecussata* and *Crassostrea gigas* respectively for the first experiment and 2.5 g of each for the second experiment. Weighing was carried out on an electrobalance to an accuracy of 0.1 mg. Daily growth rate (DGR) of the bivalves was calculated as a function of increase in wet live weight per seven day sampling period from the equation:

$$DGR = (\ln \text{final weight} - \ln \text{initial weight}) / 7$$

Culture containers and methodology were identical for both experiments with the exception of the live diet species. The juvenile bivalves were grown in 20 L recirculating downwelling systems, juveniles were kept in 30 cm diameter circular plastic 750 µm mesh screen containers suspended in 20 L buckets with exterior air lift circulators fitted. Flow rate to each container was maintained at 4 L/min. The temperature and salinity were maintained at 25 C and 34 parts per thousand (ppt) respectively. The seawater in the containers was completely changed daily with seawater filtered to 5 µm and maintained at 25 C. Live algae *Tetraselmis suecica* and *Chaetoceros sp*. were grown in semi-continuous cultures, in Guillard´s enriched f/2 medium with sterilized seawater filtered to 1 µm. Cultures were harvested every day in the exponential phase of growth in volumes dependent on the division rates of the algae.

Tests were run using a feed ration calculated at the begining of each week from the live wet weight biomass of each container as a 50% dry weight of food to wet weight biomass of bivalves per week. The daily ration was one seventh of this value fed in two doses, one in the morning and one eight hours later. For the live algal diets the volume of culture fed was taken from the cell density of the algae culture calculated from haemocytometer counts. The dry weights of the algae were calculated by filtering a measured volume of algal culture of known cell density through Whatman GF/C 57 mm glass fibre filter discs, washing with 4% ammonium formate solution, then drying to a constant weight at 65 C. The dry cell weights were found to be 200 pg/cell for *Tetraselmis suecica* and 120 pg/cell for *Chaetoceros sp.* In the first experiment the live algae component used was *Tetraselmis suecica,* in the second experiment the live algae component used was an equal mixture by dry cells weights of *Tetraselmis suecica* and *Chaetoceros sp.* The required amount of the spray-dried algae for the substitution of the live diets was first weighed then mixed with filtered seawater for 30 sec. in a domestic blender.

RESULTS

The results of the first experiment show that *Schizchytrium sp.* is capable of sustaining significantly better growth than controls in juvenile *T. semidecussata* at up to 80% algae substitution when *Tetraselmis suecica* was the only live alge feed (Table 1). Significantly higher growth over *T. suecica* fed controls was also obtained with *C. gigas* juveniles at the 40% substitution (Table 2). Although *T. suecica* has been noted as a moderate to good food for juvenile bivalves (Laing and Verdugo 1991), our experiments show this species, cultured under our conditions, to be of poor nutritional quality as indicated by the slow growth of the control animals (Fig. 1). Other investigators have found *T. suecica* to have a low food value in bivalve feeding experiments possibley due to the presence of a rigid cell wall which may complicate digestion (Cordero and Voltolino 1994).

TABLE 1

95 % Tukey analysis for *T. semidecussata* first experiment

Level	Count	Average	Homogenous groups
1 control	2	.0121500	X
3 80% replacement	2	.0201850	X
2 40% replacement	2	.0209750	X

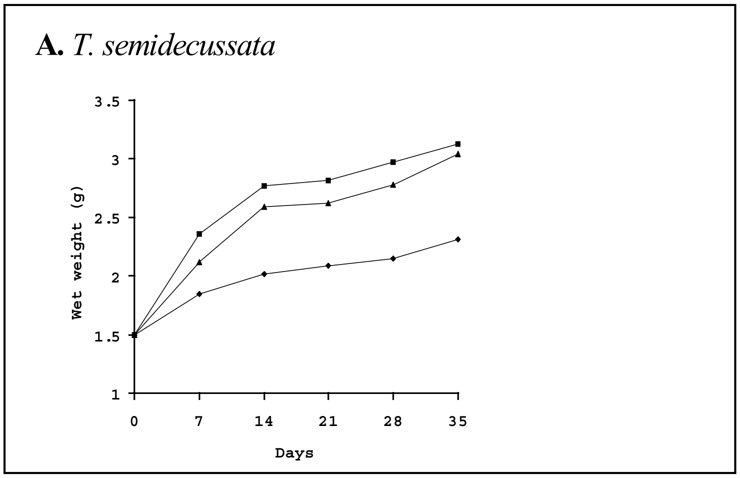
contrast	difference	+/-	limits
1 - 2	-0.00883		0.00506 *
1 - 3	-0.00804		0.00506 *
2 - 3	0.00079		0.00506 (ns)

TABLE 2

95 % Tukey analysis for *C.gigas* first experiment

Level	Count	Average	Homogenous groups
1 control	2	.0060250	X
3 80% replacement	2	.0161750	X
2 40% replacement	2	.0412250	X

contrast	difference	+/-	limits
1 - 2	-0.03520		0.01305 *
1 - 3	-0.01015		0.01305 (ns)
2 - 3	0.02505		0.01305 *



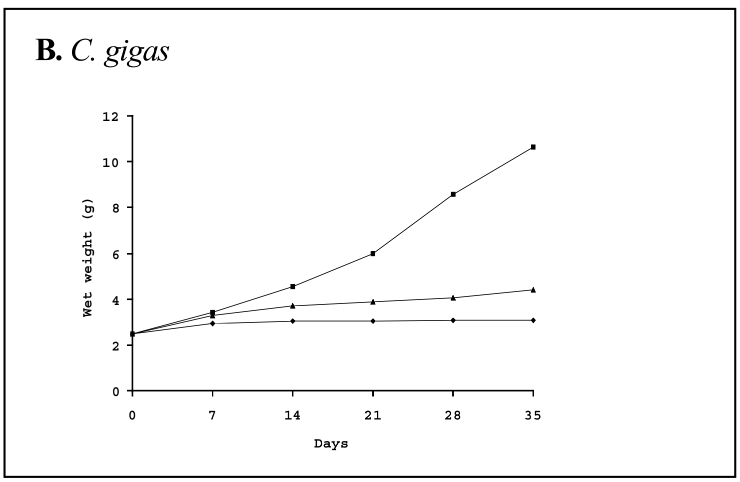


Fig. 1. Growth of *T. semidecussata* (A) and *C. gigas* (B) juveniles fed live *T. suecica* (-◆-) 40% replacement (-■-) or 80% replacement (-▲-).

The growth rate of both *C. gigas* and *T. semidecussata* controls increased by a factor of 6.3 with the mixed *T. suecica* and *Chaetocerous sp*. diet in the second experiment over the *T. suecica* alone diet in the first experiment (Fig. 2). The growth rate *of T. semidecussata* at the 40% *Schizochytrium sp.* replacement was not significantly different than control, however, all other levels of replacement for both species were significantly lower than controls (Tables 3 & 4). Growth rates of both *T. semidecussata* and *C. gigas* at the 40% substitution were significantly better than with the 80% replacement. Growth of *T. semidecussata* at the 40%. replacement was 86% of the control while the growth rate of *C. gigas* was 73% of the mixed diet control.

TABLE 3

95 % Tukey analysis for *T. semidecussatas* second experiment

Level	Count	Average	Homogenous groups
1 control	2	.0681000	X
3 80% replacement	2	.0406000	X
2 40% replacement	2	.0412250	X

contrast	difference	+/-	limits
1 - 2	0.00525		0.00620 (ns)
1 - 3	0.02750		0.01305 *
2 - 3	0.02225		0.01305 *

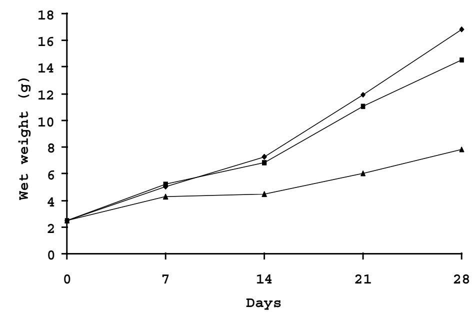
TABLE 4

95 % Tukey analysis for *C.gigas* second experiment

Level	Count	Average	Homogenous groups
1 control	2	.0812500	X
3 80% replacement	2	.0364000	X
2 40% replacement	2	.0700000	X

contrast	difference	+/-	limits
1 - 2	0.01125		0.00440 *
1 - 3	0.04485		0.00440 *
2 - 3	0.03360		0.00440 *

A. *T. semidecussata*



B. *C. gigas*

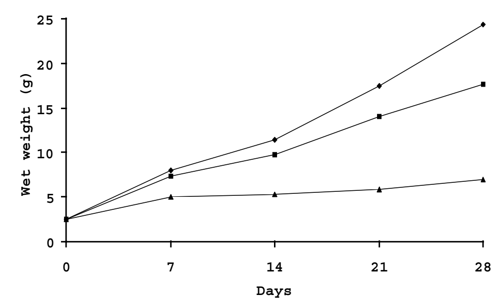


Fig. 2. Growth of *T. semidecussata* (A) and *C. gigas* (B) fed live *T. suecica* and *Chaetocerous* sp. (-◆-) 40% replacement (-■-) or 80% replacement (-▲-).

DISCUSSION

The growth rates of both *T. semidecussata* and *C. gigas* in the second experiment at 40% and 80% *Schizochytrium sp.* replacement were significantly greater than control growth rates in the first experiment. This would seem to indicate a magnification of the utility of spray-dried diet replacement in the presence of low to poor live algae sources. Due to the high cost of production, it is unlikely that most commercial facilities would be able to provide the amounts of high quality select live algae species used in the second esperiment.The costs for culturing live algae for aquatic animals have been reported by investigators to vary between US$20.00/kg dry weight and US$594.00/kg dry weight, depending on the location and size of the culture facility (Donaldson 1981, Laing and Millican 1992). The greater number of published cost estimates for live algae production at bivalve facilities are around US$200.0/kg dry weight (Walsh 1987, Walne 1976, Coutteau and Sorgeloos 1992). If these live algae production costs are only marginally accurate for today's bivalve nursery facilities, then there are realistic economic savings to be realized with *Schizochytrium sp.* replacement for live algae. For small bivalve hatcheries or larvae setting facilities using algae paste or naturally available algae, the economics may prove greater yet. These cost reductions may be further amplified when consideration is given to capitalizing hatchery live algae production systems for new start up or existing facility expansion.

These experiments are preliminary and limited to only two species of commercial bivalves. In spite of the efforts to obtain homogenous mixing of the *Schizochytrium sp.* in the containers, some settling was observed which may have affected the results. Undoubtedly, the improved mixing from well engineered upwelling systems characteristic of many commercial bivalve nursery facilities will alleviate this problem.

ACKNOWLEDGEMENTS

The authors wish to thank the following people who made this work possible: Dr. Carlos Martinez Palacios for support and use of the C.I.A.D. facilities, Mazatlan, Mexico; Dr. Dominico Voltolina, I.C.M.L, Mazatlan, Mexico, who so kindly provided all the algae used in the study; Mr. Ted Kuiper of Kuiper Mariculture, Eureka, CA., who supplied the bivalves; and Aquafauna Bio-Marine, Hawthorne, CA., who povided the Algamac-2000.

LITERATURE CITED

Albentosa, M.E., E. Naessens, P. Leger, P. Coutteau, P. Lavens, and P. Sorgeloos. 1989. Promising results in the seed culturing of the manila clam *Tapes semidecussata* with a manipulated yeast product as a partial substitute for algae. p. 7-8. In: Aquaculture Europe '89, Bordeaux, France, Oct. 2-4, 1989. Short Communications and abstracts. Bilard, R. and N. De Pauw (Comp.). European Aquaculture Society Special Publication 10, Bredene, Belgium.

Anderson, G. J. M.B. Miller, and K.K. Chew. 1982. A guide to manila clam aquaculture in Puget Sound. Washington Sea Grant Publication No. WSG-82-4. 45 p.

Bayes, J.C. 1981. Forced upwelling nurseries for oysters and clams using impounded water systems. In: C. Claus, N. De Pauw and E. Jaspers (Eds.), Nursery Culturing of Bivalve

Molluscs. Special Publication European Mariculture Society, Bredene, Belgium, 7: 73-83.

Boeing, P.L. 1986. Nursery culture of bivalve molluscs, with a report on preliminary supplementary feeding trail with microcapsules. Paper presented at the 1st Ineramerican

Congress of Aquaculture, Salvadore, Bahia, Brazil, Sept. 4-8, 1986.

Boeing, P.L. 1992. Polyculture of hard clam (*Tapes semidecussata*) with white shrimp (*Penaeus vannamei*) in shrimp ponds. Project E3-D Regional Program of Technical Co-operation in Fisheries. CEE-PEC-ALA/87/21. Guayaquil, Ecuador.

Cordero, B., and D. Voltolina. 1994. Growth of *Mytilus galloprovencialis* fed with four microalgae and two feeding regimes. Journal of the World Aquaculture Society 25: 471-476.

Coutteau, P., and P. Sorgeloos. 1992. The use of algal substitutes and the requirement for live algae in the hatchery and nursery rearing of bivalve molluscs: an international survey. Journal of Shellfish Research 11: 467-476.

Curatolo, A., M.J. Ryan, and J.P. Mercer. 1993. An evaluation of the performance of manila clam spat (*Tapes philippinarum*) fed on different rations of spray-dried algae (*Tetraselmis suecica*). Aquaculture 112: 179-186.

Helm, M.M. and S. Hancock. 1990. Growth of small bivalve spat on heterotrophically grown spray-dried algae. In: World Aquaculture '90. June 10-14, 1990. Halifax, NS Canada, Book of Abstracts IMPRICO, Quebec, Canada, pp 65.

Hidu, H., and R. Ukeles. 1962. Dried unicellular algae as food for larvae of the hard-shell clam, *Mercenaria mercenaria*. Proceedings of the National Shellfisheries Association 53: 85-101.

Jones, D.A., J.P. Mumford, and P.G. Gabbot. 1974. Microcapsules as artificial food particles for aquatic feeders. Nature 247: 233-239.

Jones, D. A., M.S. Kamarundin and L. Le Vay. 1993. The potential for replacement of live feeds in larval culture. Journal of the World Aquaculture Society 24: 199-210.

Laing, I., A.R. Child and A. Janke. 1990. Nutritional value of dried algal diets for larvae of manila clam (*Tapes philippinarum*). Journal of the Marine Biological Association of the United Kingdom 70: 1-12.

Laing, I. and P.F. Millican. 1991. Dried-algae diets and indor nursery cultivation of Manila clam juveniles. Aquaculture 95: 75-87.

Laing, I. and P.F. Millican 1992. Indoor nursery cultivation of juvenile bivalve molluscs using diets of dried algae. Aquaculture 102: 231-243.

Laing, I., and C.G. Verdugo. 1991. Nutritional value of spray-dried *Tetraselmis suecica* for juvenile bivalves. Aquaculture 92: 207-218.

Langdon, C.J., D.M. Levine and D.A. Jones. 1985. Microparticulate feeds for marine suspension feeders. Journal of Microencapsulation 2(1): 1-11.

Langdon, C.J. and C.A. Siegfried. 1984 Progress in the development of artificial diets for bivalve feeders. Aquaculture 39: 135-153.

Langdon, C.J. and M.J.Waldock. 1981. The effect of algal and artificial diets on the growth and fatty acid composition of *Crassostrea gigas* spat. Journal of the Marine Biological Association of the United Kingdom 61: 431-448.

Meyers, J.A. and R.N. Boisvert. 1990. The economics of producing algae and bivalve seed in hatcheries. Aquaculture 86: 163-179.

Rosenberry, R. 1996. ALGAMAC-2000. Shrimp News International 21(1): 4.

Urban, E.R. and C.J. Langdon. 1974. Reduction in costs of diets for the american oyster, *Crassostrea virginica* (Gmelin), by the use of non-algal supplements. Aquaculture 38: 277-294.

Walsh, D.T., C.A. Withstandley, R.A. Kraus, and E.J. Petrovits. 1987. Mass culture of selected marine microalgae for the nursery production of bivalve seed. Journal of Shellfish Research Vol. 6: 71-77.