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The Nutritional Value of Five Species of Microalgae for Spat of the Silver-Lip Pearl Oyster, *Pinctada maxima* (Jameson) (Mollusca: Pteriidae)

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Abstract

A feeding trial was conducted to assess the nutritional value of five monospecific microalgal diets for spat of the silver-lip (or gold-lip) pearl oyster, *Pinctada maxima*. The five species tested were *Isochrysis* aff. *galbana* (T-ISO), *Pavlova lutheri*, *Chaetoceros muelleri*, *Chaetoceros calcitrans* and *Tetraselmis suecica*. Spat were 75-d old at the start of the growth trial which ran for 21 d. Pearl oyster spat fed *C. muelleri* showed the largest increase in ash-free dry weight (organic content), which was significantly greater ($P<0.05$) than for any other species. The mean ash-free dry weight (AFDW) of spat fed *T. suecica* and T-ISO did not differ significantly from each other, but were significantly greater than for spat fed *C. calcitrans* and *P. lutheri* ($P<0.05$). The final AFDW of spat fed *P. lutheri* was not significantly different from that of unfed spat ($P>0.05$). Differences in the food value of the five species of algae could not be explained by their reported nutrient composition alone. The results illustrate the importance of experimental testing of algal diets for bivalve spat rather than sole reliance on published nutritional values.

Introduction

Providing an adequate diet for the early life stages of bivalves is essential to hatchery success. Despite efforts to develop artificial alternatives (Langdon and Siegfried 1984; Chu et al. 1987; Southgate et al. 1992), cultured microalgae remain a critical resource for commercial rearing of marine animals (Brown and Jeffrey 1992). The nutritional value of a given species of microalgae depends on the nature and composition of its biochemical constituents (Whyte et al. 1990) and its physical suitability (Rose and Baker 1994). There is considerable variability in the ability of different algal species to support adequate growth of bivalves (Brown and Jeffrey 1992).

Hatchery production of the pearl oyster *Pinctada maxima* is firmly established in Australia and Southeast Asia (Gervis and Sims 1992; O'Sullivan 1994; Rose 1994). There is, however, very little published information on the nutritional requirements of *P. maxima* and the suitability of different species of cultured microalgae for this species. Minaur (1969) reported on comparative feeding trials with larvae of *P. maxima* and, although there are a number of reports on rearing *P. maxima* spat, (Tanaka and Kumeta 1981; Rose 1990; Rose and Baker 1994), no information on the nutritional value of various microalgal species has yet been published. There is, however, a plethora of information regarding the nutritive value of microalgal species for larvae and spat of other bivalves such as the Pacific oyster *Crassostrea gigas* (Langdon and Waldock 1981; Laing and Verdugo 1991; Laing and Millicen 1992; Thompson et al. 1993), the European flat oyster *Ostrea edulis* (Walne 1963, 1970; Laing and Verdugo 1991), the Sydney rock oyster *Saccostrea commercialis* (O'Connor et al. 1992); the clams *Mercenaria mercenaria* (Laing and Verdugo 1991; Wikfors et al. 1992) and *Tapes phillipinarum* (Laing and Millican 1991; Laing and Verdugo 1991),the mussel *Mytilus galloprovincialis* (Fidalgo et al. 1994) and the scallops *Patinopecten yessoensis* (Whyte et al. 1989) and *Crassadoma gigantea* (Whyte et al. 1990).

Tropical aquaculture hatcheries are still largely reliant on species of microalgae isolated from cooler, temperate waters (Jeffrey et al. 1992). Hatchery propagation of *P. maxima* in Australia typically uses isolates from temperate waters cultured at cooler than ambient temperature (O'Sullivan 1994). This in itself may be a problem when microalgae cultured at a relatively low temperature are fed to bivalve larvae or spat cultured at a higher water temperature. For example, Tanaka and Inoha (1970) did not recommend the use of *Pavlova lutheri* as a food for the black-lip pearl oyster (*Pinctada margaritifera*) because when the microalgae were taken from an optimum growing temperature of 20°C and introduced into larval culture vessels at 28-30°C, it quickly died. However, this alga is still widely used in commercial tropical pearl oyster hatcheries (Gervis and Sims 1992) and was recommended as a food for *P. maxima* larvae by Minaur (1969).

This study assessed five species of microalgae for their nutritional value for pearl oyster (*P. maxima*) spat. The five species were selected on the basis of their ease of culture under tropical conditions and their reported nutritional values; they were the golden brown flagellates *Isochrysis* aff. *galbana* (clone T-ISO) and *Pavlova lutheri*, the diatoms *Chaetoceros muelleri* and *C. calcitrans,* and the green flagellate *Tetraselmis suecica*. T-ISO and *C. muelleri* are rated as good sub-tropical to tropical species (temperature range 15-30°C), *P. lutheri* as a good sub-tropical species (10-25°C) and *C. calcitrans* and *T. suecica* as excellent universal species (10-30°C) (Jeffrey et al. 1992). All five species have previously been used at 25±1°C with moderate to excellent results with Sydney rock oyster *Saccostrea commercialis* spat (O'Connor et al. 1992).

Materials and Methods

Batches of 13 hatchery-reared *P. maxima* spat were held in 1-mm mesh baskets (100 x 70 x 30 mm) and individual baskets were suspended in 750-ml aerated plastic aquaria. Each aquarium contained 1 µm filtered U.V. sterilized seawater (36‰). At the start of the growth trial, spat were 75-d old with a mean shell length and wet weight of 4.48 ± 0.03 mm and 5.59 ± 0.11 mg (mean ± SE, n = 312), respectively. Fifteen individuals were dried at 50°C for 48 h and then heated at 500°C for 4 h to determine initial ash content. The ash-free dry weight (AFDW) was calculated as the difference between dry weight and ash weight. The mean AFDW at the start of the experiment was 0.25 mg.

Five species of microalgae were assessed for their nutritional value to *P. maxima* spat: *Isochrysis* aff. *galbana* (clone T-ISO, CS-176), *Pavlova lutheri* (CS-182), *Chaetoceros calcitrans* (CS-178), *C. muelleri* (formerly *C. gracillis*, CS-176) and *Tetraselmis suecica* (CS-187). Algal stock (starters) cultures were obtained from CSIRO, Hobart, Australia. Algae were cultured in 2-l borosilicate flasks using f medium (Guillard 1972) in 0.2 µm filtered seawater. Cultures were maintained at 24 ± 0.5°C using a 14:10 h light: dark cycle. Cultures were harvested for feeding to spat during the exponential or log growth phase. Each species was fed on an equal dry weight basis, using previously published dry weight values (Nell and O'Connor, 1991; O'Connor et al. 1992), at an initial daily ration equivalent to 80,000 T-ISO cells ml^{-1} d^{-1}. This amount was increased every 4 d by 5,000 cells ml^{-1} d^{-1} to approximate feeding regimes used for commercial production of *P. maxima* spat. An unfed control treatment was also included and each treatment was assessed in triplicate.

The experiment was terminated after 21 d. Shell length was determined for individual spat. Wet weight and AFDW were determined by weighing each replicate group of spat and dividing by the number of surviving individuals. Length and weight data were compared using one-way ANOVA (Sokal and Rohlf 1981) with means compared pairwise using Fisher's protected least significant difference (PLSD) test from the Statview statistical program, version 4.02, for Macintosh computers (Abacus Concepts, StatView, 1992). Homogeneity of variance was confirmed with Cochran's test (Snedecore and Cochran 1967).

Results

Survival was generally high during the experiment (>73%) with the exception of one replicate fed *C. calcitrans*. This replicate suffered high mortality (62%) in the final week of the trial with only five live animals retrieved. The reason for this mortality was unclear. Disregarding this replicate; there were no significant differences (P>0.05) in survival between treatments.

Growth (shell length, wet weight and AFDW) of spat fed *C. muelleri* was significantly greater (P<0.05) than for any other species tested (Fig. 1). Shell length and wet weight did not differ significantly (P>0.05) between

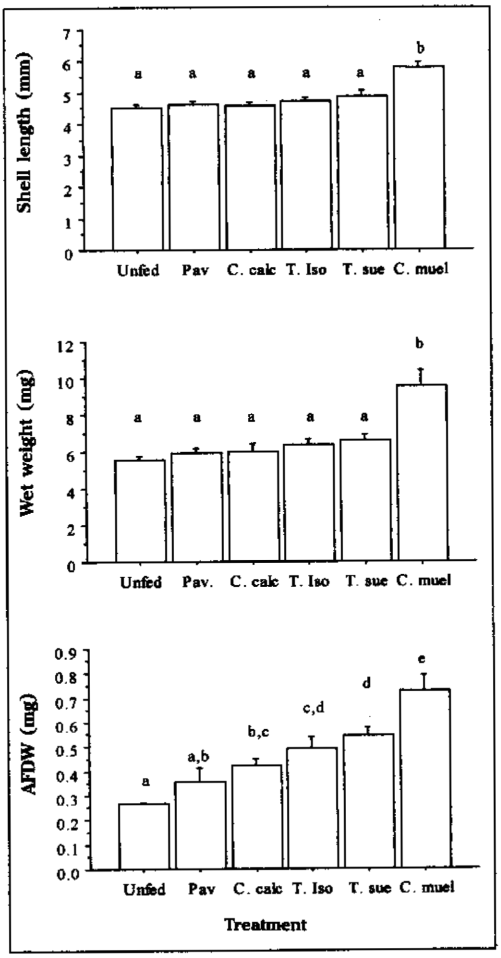

Fig. 1. Mean (±SE, n = 4) shell length, wet weight and ash-free dry weight (AFDW) of *P. maxima* spat fed five species of microalgae after a 21-d growth trial. Initial = AFDW before growth trial, Unfed = unfed control, Pav = *P. lutheri*, C. calc. = C. calcitrans, T.Iso = T- ISO, T.sue. = *T. suecica* and C.muel. = *C. muelleri*. Values with the same superscript are not significantly different (P>0.05).

spat fed either *P. lutheri*, *C. calcitrans*, T-ISO or *T. suecica* and the unfed control group. There was no significant difference (P>0.05) in final AFDW between the unfed control and spat fed *P. lutheri*. Spat fed any of the other microalgal species had significantly greater AFDW than the unfed controls (P<0.05). Final AFDW of spat did not differ significantly (P>0.05) between *C. calcitrans* and *P. lutheri* or between T. ISO and *T. suecica* (Fig. 1).

Discussion

Of the five species of microalgae species assessed, *C. muelleri* supported the greatest increase in shell length, wet weight and AFDW of *P. maxima* spat. These results support those of similar growth trials with bivalves. For example, Enright et al. (1986) ranked *C. gracillis* (=*C. muelleri*) as the best single species diet when compared with 16 other microalgal species. O'Connor et al. (1992) rated *C. muelleri* very highly as a diet for Sydney rock oyster (*Saccostrea commercialis*) spat, and not significantly different (P>0.05) from the best performing monospecific diet, *Skeletenoma costatum*.

The result obtained for *T. suecica* is interesting as there are conflicting reports of its nutritional value. Walne (1970) reported that "...species of *Tetraselmis* are of outstanding value as food for juvenile bivalves"; however, Langdon and Waldock (1981) and Laing and Verdugo (1991), described *T. suecica* as being of "moderate food value" to juvenile bivalves. Epifanio (1979) and Laing and Verdugo (1991) suggested that the food value of *T. suecica* is enhanced when fed as part of a mixed species diet. Poor performance of *T. suecica* in other feeding trials has been ascribed to difficulties met by spat in digesting the theca of this alga (Epifanio 1979). Indeed, Heasman et al. (in press) found that *T. suecica* was poorly ingested and not digested by adult scallops, *Pecten fumatus*. Similar feeding difficulties associated with *T. suecica* were not apparent in the present study. Clearly, the experimentally derived nutritional rating of a microalgal species depends to a great degree on what it is compared with and to which species it is fed.

The remaining species produced moderate to poor growth. The low growth rates of spat fed *C. calcitrans* and *P. lutheri* are surprising, as both are generally highly rated foods for bivalves (Brown et al. 1989). In a similar trial with Sydney rock oyster spat, *P. lutheri* performed moderately well and not significantly different to *T. suecica,* and *C. calcitrans* performed as well as *S. costatum*, which was the best species trialed (O'Connor et al. 1992). *C. calcitrans* was also slightly better, although not significantly so, than *C. gracillis* (= *C. muelleri*) and a great deal better than *T. suecica* (O'Connor et al. 1992). As the seawater temperature was 25±1°C in both trials, it would seem that there may be differences in the nutritional requirements of *P. maxima* spat or with the palatability of *P. lutheri* and *C. calcitrans*. As the nutritional value of microalgae varies according to growing conditions (Walne 1970), local conditions may have influenced the nutritional quality and subsequent performance of both species. The use of *P. lutheri* for the black-lip pearl oyster *P. margaritifera* was questioned by Tanaka and Inoha (1970); problems were associated with algal morbidity due to differences between algal and larval culture temperatures. However, in this study the temperature difference between the algae and the spat culture vessels was slight (1-2°C). Minaur (1969) rated *P. lutheri* and T-ISO as the best two species to feed to *P. maxima* larvae after comparing larval growth rates when fed 11 different monospecific diets. Growth of larvae was further enhanced when equal numbers of cells of *P. lutheri* and T-ISO were fed as a mixture. As has been suggested for other bivalves (O'Connor et al. 1992), there may well be differences in the nutritional requirements of *P. maxima* larvae and spat.

There are conflicting reports on the relationship between the gross biochemical composition of microalgae and their nutritional value. In a feeding trial with scallop larvae (*Patinopecten yessoensis*), the level of carbohydrate in the diet was highly correlated with larval quality (Whyte et al. 1989). *T. suecica* has over double the amount of carbohydrate of *C. calcitrans* and T-ISO and a third more than *P. lutheri* (Volkman et al. 1989). However, Wikfors et al. (1992) found that the importance of dietary carbohydrate was far less than that of protein and lipid, which were found to be the most important gross biochemical constituents for clam nutrition. *T. suecica* has relatively low amounts of dietary lipid but similar amounts of protein compared to the other species trialed (Volkman et al. 1989).

Bivalves have a dietary requirement for the long chain highly unsaturated fatty acids (HUFA) eicosapentaenoic acid (20: 5n-3; EPA) and docosahexaenoic acid (22: 6n-3; DHA) (Trider and Castell 1980; Langdon and Waldock 1981; Enright et al. 1986), and species of microalgae rich in these HUFA are generally assumed to be of high nutritional value (Brown et al. 1989). The fatty acid profiles of a number of species of microalgae originating form the same laboratory as the stock used in this study (CSIRO, Hobart), have previously been determined (Volkman et al. 1989). Volkman et al. (1989) reported significant levels of EPA in *C. calcitrans*, *C.*

gracilis (= *C. mulleri*) and *P. lutheri*, and low levels in T-ISO and *T. suecica*. The same study also reported trace amounts of DHA in *T. suecica*, small amounts (<0.8% total fatty acids) in the *Chaetoceros* species and significant levels of DHA in T-ISO and *P. lutheri*. Thus, of the species assessed in the present study, only *P. lutheri* would be expected to contain high levels of both EPA and DHA. Nevertheless, this species supported the lowest mean weight gain of *P. maxima* spat. Additionally, *T. suecica*, which would be expected to contain only trace amounts of DHA, supported a significantly better growth rate than *P. lutheri*.

Thompson et al. (1993) suggested that the nutritional value of EPA and DHA has been somewhat overrated and that there are threshold levels of these fatty acids beyond which growth does not improve. Thompson et al. (1993) reported that the fastest growing *Crassostrea gigas* oyster larvae contained relatively higher levels of the 14: 0 and 16: 0 fatty acids, and it was suggested that the nutritional value of microalgae was more closely related to saturated fatty acid composition than to HUFA content. The C16 fatty acids form a relatively high percentage (>20.0%) of the total fatty acids for *T. suecica* (Volkman et al. 1989).

This is the first study to assess single species diets for *P. maxima* spat and give an indication of suitable microalgal species to use as feed. Undoubtedly a mixed diet will produce far better growth in *P. maxima*, as has been previously demonstrated with other bivalves (Whyte et al. 1989; Whyte et al. 1990; O'Connor et al. 1992; Fidalgo et al. 1994). The results presented here are an indication of the importance of testing algal diets experimentally rather than relying solely on previously published data when selecting suitable species of microalgae as food. Finally, cultured microalgae fed to *P. maxima* spat in the hatchery are, as yet, unable to match the growth recorded in spat held at sea (Rose and Baker 1994; Taylor, unpubl. data) and considerable work is required before a truly adequate diet is developed for *P. maxima* spat.

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