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Short Communication

Evaluation of the Microalgal Substitute M-1 as a Diet for Juvenile Sydney Rock Oyster, *Saccostrea glomerata* (Gould 1850)

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Abstract

To investigate ways to reduce Sydney rock oyster, *Saccostrea glomerata* (Gould 1850) hatchery operational costs, an artificial diet "M-1" was tested for use with *S. glomerata* spat. The "M-1" diet was either fed as a sole food source or combined with fresh algae, *Chaetoceros muelleri,* at various concentrations ranging from 10% M-1 to 90% M-1 in 10% increments. Spat were fed each of the diets for 10 days and their growth and survival were compared with spat fed the current commercial hatchery diet of 50% *C. muelleri*, 25%, Tahitian *Isochrysis* aff. *galbana* and 25% *Pavlova lutheri.* A diet of solely M-1 produced comparatively poor growth, while diets containing between 30-80% M-1 produced growth comparable to that of the current commercial hatchery diet. The greatest growth rate recorded in this trial was produced by a diet of 50% M-1 and 50% *C. muelleri*. Although this diet does not overcome the need for live algal cultures, it significantly reduces the cost and complexity of the diet in comparison to that currently used for commercial *S. glomerata* production.

Introduction

While appropriately chosen microalgal diets offer sufficient nutritional value for larval and juvenile oysters, algal culture can limit production outputs and constitutes a major ongoing cost for hatcheries (Coutteau and Sorgeloos 1992; Knauer and Southgate 1997; Heasman et al. 1999). Accordingly, numerous studies have been undertaken to investigate suitable substitutes for live algal cultures as feeds for bivalves (Knauer and Southgate 1997), including formulated, artificial particulate diets (Nevejan et al. 2007).

To be of use, a successful artificial diet must comprise particles of a suitable size for filtration and digestion that are impermeable and stable in seawater but can be readily broken down by the action of digestive enzymes (Jones et al. 1974). The diet should be neutrally buoyant and cheaper to provide than algae (Numaguchi and Nell 1991).

To date several artificial dietary supplements and algal substitutes have been trialled for culture of the Sydney rock oysters, *Saccostrea glomerata* (Gould, 1850), with limited success (Nell and Wisely 1983; Numaguchi and Nell 1991, Southgate et al. 1992, Nell et al. 1996).

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Recently several new products have appeared that could be of value to bivalve production, one of those diets is M-1 (Nosan Corporation, Japan). M-1 is powder with a particle size of 10-15 µm and a refrigerated shelf life of 1 year. Research by Nosan reports feeding trials with clams, scallops and pearl oysters which have resulted in increased growth and condition index {(dried meat weight/inner shell volume) x 1000}.

This trial sought to evaluate M-1 as a substitute for live algal as a food source for *S. glomerata* spat (recently settled juvenile oysters).

Materials and Methods

One week old spat, 949±39 µm were obtained from the Port Stephens Fisheries Institute, mollusc hatchery. Fifty spat were placed into each of 36 miniature upweller systems, each held at 25 ^{o}C. Each system comprised an upweller with a 200 µm nylon screen base held in an individual 8 L plastic aquarium. Water circulation to each upweller was driven by an air-lift pump. The oysters were allowed to acclimatise for 24 h without food before the experiment began.

The experiment (with three replicates per treatment) ended 10 days later, corresponding to the period of time spat are held in the hatchery before they are large enough to be transferred to field-based nurseries and natural diets.

All treatments were fed at an algal cell density of 100,000 Tahitian *Isochrysis* aff. *galbana* (ANACC, CS-177) cells mL^{-1} day^{-1}, or its dry weight equivalent in algae and M-1. This feed rate ensured that the oysters within each treatment were being fed to satiation. All treatments were cleaned and received a complete water exchange every second day using 1 µm filtered, temperature equilibrated seawater.

At the end of the trial, spat from each replicate were collected and preserved using 10% formalin in seawater. The shell lengths (antero-posterior measurement) of 50 randomly selected spat per treatment were measured. The number of dead spat (empty shells or containing necrotic tissue) encountered in measuring 50 fresh (non preserved) spat was recorded and used to calculate percent mortality.

All algae used in this trial were cultured in 10 L polycarbonate carboys using f/2 media (Heasman et al. 1999) and harvested in late-exponential growth phase. A new culture was used every 3-4 days, with three independent cultures being used during the course of the trial. Algal cell counts were determined daily before feeding.

The M-1 diet was prepared daily by weighing the required quantity to the nearest 0.001 g and blending with 500 mL of distilled water for 10-20 s using a household blender. Between use the dry M-1 was kept refrigerated at 4 ºC. The amount of M-1 substituted for algae in each diet was calculated on the basis that each gram of M-1 was the equivalent of 2.4 x 10^{9} cells of T. *Isochrysis*.

Spat were fed one of eleven diets (Fig. 1). One diet comprised solely M-1, while a further nine diets made by combining M-1 with *Chaetoceros muelleri* (ANACC, CS-176) to prepare mixtures that ranged from 10-90% M-1 on a dry weight basis. *Chaetoceros muelleri* was selected as the diluent algae because of its productivity and reliability in culture and its value as food for *S. glomerata* spat (O'Connor et al. 1992). The final diet comprised 50% *C. muelleri*, 25% T. *Isochrysis* and 25% *Pavlova lutheri* (ANACC, CS-182) and was included as a control to allow comparison with the diet used currently for commercial *S. glomerata* spat production.

Statistical analysis

Homogeneity of variance was confirmed using the Cochran test (Winer et al. 1991) and the data was analysed using a single-factor analysis of variance (ANOVA). Mean values were compared using Tukey's honestly significant difference method (Sokal and Rohlf 1981).

Results

Significant differences were detected among growth rates of *S. glomerata* spat fed diets comprising M-1 or M-1 in combination with *C. muelleri* and the current commercial hatchery diet ($F= 14.71$; df 10,32; $P<0.0001$; Fig. 1). The greatest growth rate recorded was among spat fed a 50% M-1 diet, whereas growth equivalent of that recorded using the standard hatchery diet was observed in treatments including between 60-80% M-1 (Fig 1). The lowest growth rate observed was in spat fed 100% M-1, which was approximately 45% lower than that observed in the best performing diet (50% M-1).

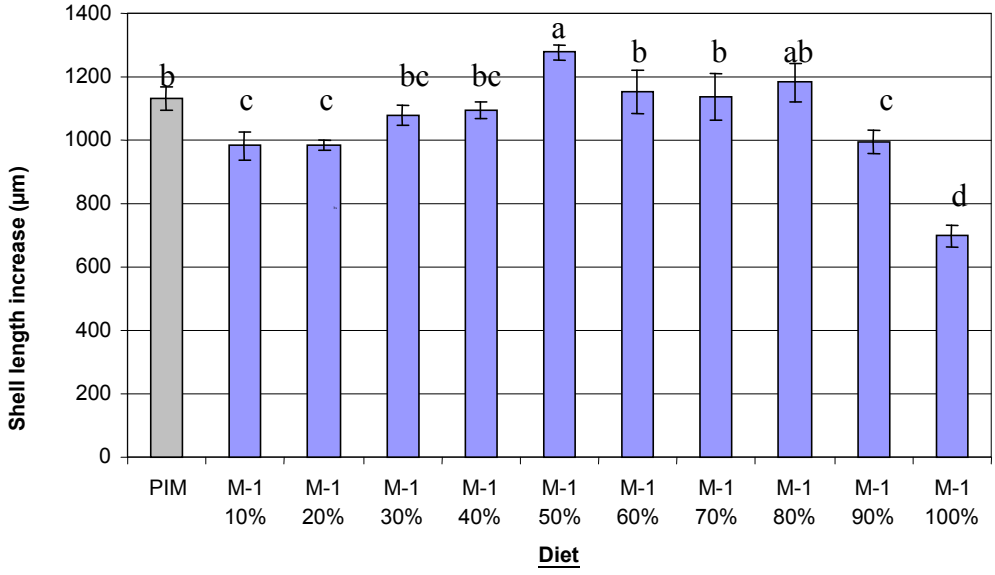

Fig. 1. A comparison of growth of *Saccostrea glomerata* spat (initial shell length 949 um) fed diets comprising the artificial diet M-1 in combination with *Chaetoceros muelleri* with the current commercial hatchery diet (PIM = 50% *C. muelleri*, 25% T. *Isochrysis* and 25% *Pavlova lutheri*).

Significant differences were also detected among survival of *S. glomerata* spat fed each diet ($F= 7.81$; df 10,32; $P<0.0001$); but, the only diet in which significant mortality was observed was the 100% M-1 treatment, with a mean mortality of 19%.

Discussion

Inclusion of the artificial diet M-1 in the diet of *S. glomerata* spat offers great promise in reducing hatchery production costs and simplifying algal production procedures. While comparatively poor growth and increased mortality was observed among spat fed solely M-1, growth better than or equal to that of the standard hatchery diet was recorded when spat were fed diets incorporating between 50-80% M-1. Even at the lower of these inclusion rates significant cost savings can accrue.

Estimates of algal demand during standard hatchery production suggest that *S. glomerata* oyster larvae consume approximately ⅓ the quantity of algae that is subsequently fed to that batch during the nursery rearing phase (W. O'Connor pers. comm.). Accordingly, the substitution of 50% of the spat diet with M-1 reduces overall algal demand for each production run by more than ⅓. Moreover, the reduction in total volume of algae required for spat production also causes a concomitant reduction in the quantity of "surplus" algae produced to account for batch failures, contamination or accidental loss (leakage of spillage). Further advantages of the use of M-1 arise from a simplification of the algal production through a reduction in the number of species required for spat production from three (*C. muelleri*, T. *Isochrysis* and *P. lutheri*) to solely *C. muelleri*. This is compounded by the fact that *C. muelleri* was in part chosen as the diluent for M-1 because of its productivity and reliability in culture.

While M-1 is not the first artificial diet to show promise for bivalve production (see Nevejan et al. 2007) it may be among the vanguard of new diets that reduce our dependence on algal culture. A useful additional step would now be to assess the use of these artificial diets in broodstock conditioning, where even greater quantities of food are often required over longer periods of time.

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