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SCIENCE DIRECT
Aquaculture 257 (2006) 491–503
Aquaculture
www.elsevier.com/locate/aqua-online

The nutritional value of seven species of tropical microalgae for black-lip pearl oyster (*Pinctada margaritifera*, L.) larvae

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Received 22 December 2005; received in revised form 12 March 2006; accepted 13 March 2006

Abstract

Recent years have seen major developments in the culture and availability of tropical microalgae as a food source for tropical bivalve species. The nutritional value of seven small (<9 μm) tropical microalgae species: two diatoms (*Chaetoceros muelleri* and *Chaetoceros* sp.); three golden-brown flagellates (*Isochrysis* sp., *Pavlova salina* and *Pavlova* sp.) and two green-flagellates (*Micromonas pusilla* and an unidentified coccoid CS-126), were analysed for carbohydrate, lipid and protein contents as well as fatty acid composition. Each species of microalgae was fed singly to early (D-stage veliger) and later (umbo stage veliger) stage larvae of the black-lip pearl oyster, *Pinctada margaritifera*. Highest survival of D-stage larvae over the 10-day experiment was recorded for those fed *Pavlova* sp. (CS-50). Greatest shell growth was shown by D-stage larvae fed the golden-flagellates *Pavlova* sp. (CS-50) and *Pav. salina*. Based on growth of D-stage larvae, the microalgae could be divided into three groups: (1) larvae fed *Pav. salina* and *Pavlova* sp. showed significantly greater growth than those fed other microalgae; (2) those fed *Isochrysis* sp., *C. muelleri* and *M. pusilla* showed significantly greater growth than unfed larvae; and (3) larvae fed *Chaetoceros* sp. and CS-126 did not grow at a rate greater than unfed larvae. Growth of D-stage veliger larvae was significantly correlated with carbohydrate, lipid and protein content of microalgae and with levels of dietary polyunsaturated fatty acid, specifically DHA ($r=0.829$, $P=0.021$). In a second experiment survival of umbo-stage larvae (including the unfed control) did not differ significantly between treatments ($P<0.05$) after 8 days of culture. Larvae fed *Pavlova* sp. and *Pav. salina* showed the greatest incremental growth increases, but these were not significantly greater than those of larvae fed TISO and *C. muelleri* ($P>0.05$). Growth of umbo-stage larvae fed *M. pusilla*, *Chaetoceros* sp. and the Prasinophyta sp. (CS-126) did not differ significantly from that of unfed larvae ($P<0.05$). This study is the first comprehensive assessment of the nutritional value of tropical microalgae species for pearl oyster larvae. The results provide a basis for development of more effective larval culture techniques by identifying microalgae supporting good growth of *P. margaritifera* larvae of different ages.

Keywords: Microalgae; Nutritional; Pearl oyster; *Pinctada margaritifera*; Larvae; Fatty acid; Gross biochemical composition

1. Introduction

Microalgae are the major food source for bivalves (Knauer and Southgate, 1999). Reflecting that the majority of bivalve aquaculture concerns temperate species, the majority of the microalgae species so far assessed for their nutritional value for bivalves have

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doi:10.1016/j.aquaculture.2006.03.022

been of temperate origin (e.g. Delaunay et al., 1993; Utting and Millican, 1997; Brown et al., 1998; Leonardos and Lucas, 2000). Interest in the culture of tropical microalgae species (e.g. Renaud and Parry, 1994; Renaud et al., 1994) has increased over recent years in response to the growth of tropical mariculture and the resulting need for nutritious microalgae that are tolerant to tropical culture conditions. However, despite improvement in the availability of tropical microalgae for mariculture, relatively little information is available on their nutritional value for bivalve larvae.

Biochemical composition is a major factor in determining the nutritive quality of microalgae and their utility as food for bivalves. Much of the research in this field has been conducted with juvenile or adult bivalves (e.g. Rodhouse et al., 1983; Taylor et al., 1997) and has focused on the highly unsaturated fatty acid (HUFA) profiles of microalgae as an indicator of nutritional quality (e.g. Albentosa et al., 1996). The link between the presence of HUFA in microalgae, specifically eicosapentaenoic (EPA, 20:5*n*-3) and docosahexaenoic (DHA, 22:6*n*-3), and high nutritional value is now well established (Knauer and Southgate, 1999; Volkman and Brown, 2005). Fewer studies in this field have been conducted with bivalve larvae and, while dietary HUFA have been shown to be similarly important (Chu and Webb, 1984; Marty et al., 1992; Pernet and Tremblay, 2004), other studies have reported a negative influence of polyunsaturated fatty acids (PUFA) and *n*-3 fatty acids on growth of bivalve larvae and positive correlations between the levels of saturated fatty acids and larval growth (Thompson et al., 1996; Leonardos and Lucas, 2000). Furthermore, Whyte (1987) reported a high correlation between dietary carbohydrate level and the quality of scallop, *Crassodoma gigantea*, larvae, where carbohydrate was thought to spare other major nutrients for tissue synthesis.

While there is limited and somewhat conflicting information on the relationship between the nutrient composition of microalgae and their nutritional value for bivalve larvae, a number of recent studies have used biochemically-based models to simulate growth and survival of bivalve larvae under varying conditions (Powell et al., 2002, 2004; Hofmann et al., 2004). Models suggested that increasing survival of oyster, *Crassostrea gigas*, larvae was associated with low dietary protein and high dietary lipid levels (Powell et al., 2002, 2004) and that relatively high levels of carbohydrate are important for larvae in preparation for settlement (Powell et al., 2004). Furthermore, Hofmann et al. (2004) suggested that the quality of microalgae, as a factor influencing growth and survival of *C. gigas* larvae, was best described by the ratio of dietary protein to the sum of lipid and carbohydrate. Knowledge of the biochemical compositions of microalgae, the presence of key nutrients, and models based on biochemical changes in larvae, provide a useful guide to the potential nutritional value of microalgae and larval nutrient requirements. However, variation in the results obtained in growth trials with bivalve larvae and differences in cytomorphological characteristics of microalgae, such as cell wall thickness and resulting digestibility, require elucidation of their true nutritional value in growth trials with the target species.

Cultured pearls provide the basis for major mariculture industries in the Asia–Pacific region (Gervis and Sims, 1992; Fassler, 1997). The majority of cultured pearl production occurs in tropical regions using the silver- or gold-lip pearl oyster, *Pinctada maxima*, and the black-lip pearl oyster, *P. margaritifera*. Over recent years, hatchery production has become an increasingly important source of pearl oyster stock for the pearling industry (Gervis and Sims, 1992), and hatchery culture methods are well established for both *P. maxima* and *P. margaritifera* (Rose and Baker, 1994; Southgate and Beer, 1997). Early research into the artificial rearing of tropical pearl oyster larvae highlighted problems when temperate microalgae species were used as a food source for larvae cultured at tropical water temperatures (Minaur, 1969; Tanaka et al., 1970). Although the greater suitability of tropical microalgae for rearing larvae of tropical pearl oysters was proposed by Minaur (1969), further study in this field has been limited. Increasing availability of tropical microalgae over recent years has, however, allowed some species to be assessed for their physical suitability as a food source for pearl oyster larvae (Doroudi et al., 2003) as well as their nutritional value (Southgate et al., 1998). However, no prior study has determined the nutritional value of tropical microalgae for pearl oyster larvae on the basis of their nutrient compositions.

This study assessed the nutritional value of seven selected species of tropical microalgae for larvae of *P. margaritifera*. Detailed biochemical analyses of these species were undertaken in an effort to identify important nutritional components for *P. margaritifera* larvae and nutrients imparting high nutritional value to microalgae.

2. Material and methods

2.1. Microalgae cultures

The microalgae used in this study were obtained from CSIRO Marine Laboratories in Hobart, Tasmania