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Nutritional Value of Binary Microalgal Diets for Larvae of the Blacklip Pearl Oyster *Pinctada margaritifera* (L.)

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Abstract

This paper reports on two experiments to assess the nutritional value of cultured microalgae for larvae of the blacklip pearl oyster, *Pinctada margaritifera*. In the first experiment, two binary diets were assessed and *Isochrysis* sp. (T-ISO) was administered in combination with either *Pavlova salina* or the diatom *Chaetoceros muelleri*. The second experiment assessed the nutritional value of the individual species of microalgae from the best binary diet used in the first experiment (T-ISO and *C. muelleri*). Larvae fed T-ISO/*C. muelleri* in the first experiment showed significantly better growth and survival ($P < 0.05$) than those fed T-ISO/*Pav. salina* after 20 days of feeding. Administering the microalgae from the best binary diet separately, showed that *C. muelleri* alone is unsuitable for rearing D-stage *P. margaritifera* larvae. Larvae fed with T-ISO showed significantly better growth and survival ($P < 0.05$) than those fed *C. muelleri* at the end of 9 days of feeding and the slower growth rate of larvae fed *C. muelleri* was apparent from day 5. The mean antero-posterior shell measurement (APM) of larvae fed *C. muelleri* at day 5 (88.33 ± 1.95 μm) was significantly smaller ($P < 0.05$) than that of the larvae fed T-ISO (94.33 ± 4.47 μm). The data generated in this study will be valuable in the further refinement of hatchery culture techniques for *P. margaritifera* throughout the Indo-Pacific region.

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Introduction

Over recent years, there has been a major increase in the use of hatchery-reared juveniles in the cultured pearl industry (Gervis and Sims 1992; Rose and Baker 1994; Southgate and Beer 1997). The blacklip pearl oyster, *Pinctada margaritifera* (L.), supports cultured pearl industries in French Polynesia and the Cook Islands (Fassler 1995) and is the subject of experimental or smaller scale pearl culture operations in other Pacific countries such as Kiribati, Fiji, Solomon Islands and Marshall Islands (Clarke et al. 1996; Friedman and Bell 1996; Southgate 1996; Fassler 2002). As part of this development, a pilot hatchery was established on the island of Tarawa in the Republic of Kiribati in 1995. This hatchery now produces commercial quantities of *P. margaritifera* spat on a routine basis. However, although reliable hatchery culture techniques have been described for *P. margaritifera* (Southgate and Beer 1997), many areas of hatchery culture require further research to optimise larval culture techniques; one such area is larval nutrition.

Very little is known about the nutritional requirements of pearl oyster larvae (Southgate et al. 1998). However, information on the nutritional value of a small number of temperate (Tanaka et al. 1970) and tropical species of microalgae (Southgate et al. 1998; Martinez-Fernandez et al. 2006) for the larvae of *P. margaritifera* has been reported. Southgate et al. (1998) reported the golden-brown flagellates, *Isochrysis* (Tahitian) (clone T-ISO) and *Pavlova salina,* to be of high nutritional value for *P. margaritifera* larvae. They suggested that a mixture of T-ISO and *Pav. salina* might provide a good diet for *P. margaritifera* larvae; however, this has not been determined. In a more recent study, Martinez-Fernandez et al. (2006) reported high nutritional value of *Pavlova* spp. for both D-stage and umbone *P. margaritifera* larvae. Furthermore, the nutritional value of diatoms (*Chaetoceros* spp.) was significantly lower than that of *Pavlova* spp. when fed to D-stage *P. margaritifera* larvae, but assumed greater nutritional value when fed to older umbone larvae (Martinez-Fernandez et al. 2006). While it is generally accepted that a microalgal diet composed of more than one species provides a better nutritional 'balance' for bivalves (Webb and Chu 1983), there is a paucity of information on the nutritional value of binary microalgal diets for pearl oyster larvae.

This study reports on two experiments, which assessed the nutritional value of microalgae for *P. margaritifera* larvae under hatchery conditions in Kiribati. In the first, the nutritional value of two binary microal-

gal diets was assessed; each diet contained T-ISO in combination with either *Pav. salina* or the diatom *Chaetoceros muelleri*. In the second experiment, the nutritional value of individual species from the best binary diet used above (T-ISO and *C. muelleri*) was assessed. All three species of microalgae used in this study are readily ingested by *P. margaritifera* larvae of all ages (Doroudi et al. 2003).

Materials and Methods

Two feeding experiments were conducted at the Ministry of Fisheries and Marine Resource Development (Fisheries Division) pearl oyster hatchery at Tanaea on Tarawa atoll in Kiribati. In both experiments, *P. margaritifera* adults were induced to spawn using thermal stimulation and eggs were incubated according to the methods of Southgate and Beer (1997). After 24 h the resulting D-stage veliger larvae were stocked at a density of 2 mL^{-1} into 60 L tanks containing gently aerated 1 μm filtered seawater. Water in the tanks was changed every second day for the first 11 days when larvae were collected on a 37 μm sieve mesh (Southgate and Beer 1997). From day 13 onwards, 100% water changes were conducted every third day with 50% water exchanges on all other days. During the main water change, replicate samples of larvae were counted to estimate the total number of larvae in each tank, and a random sample of 30 larvae were measured for antero-posterior shell length (APM). Larval culture tanks were maintained in an outdoor covered hatchery area and subjected to the ambient temperature regime. Water temperature in larval culture tanks ranged between 29°C and 31°C during the culture period.

Microalgae culture

Microalgae used in this study were obtained from CSIRO Marine Laboratories in Hobart, Tasmania (Australia). The three microalgae species used were *Chaetoceros muelleri* [CS-176], *Pavlova salina* [CS-49] and *Isochrysis* (Clone T-ISO) [CS-177] (codes refer to CSIRO catalogue codes). Microalgae were batch cultured in replicate 5 L glass flasks in 0.45 μm filtered and UV treated seawater using Walne's nutrient medium (Walne 1974). Illumination was provided by cool white fluorescent lights on a 10 h L – 14 h D photoperiod and cultures were maintained at 26 ± 1°C. Between 3 and 5 replicate cultures were established for each species and algae were harvested from them in a sequential manner during the late exponential growth phase. Cell dimensions for *C. muelleri* (excluding

spines), *Pav. salina* and T-ISO were 5 x 8 μm, 5 x 5 μm and 3 x 5 μm, respectively.

Experiments assessing the nutritional value of microalgae

In Experiment 1, two binary feeding regimes were assessed for *P. margaritifera* larvae: (1) T-ISO and *Pav. salina*; and (2) T-ISO and *C. muelleri*. Each combination was fed as an equal mixture (1:1 on a cell number basis) of each species at the rations shown in table 1. Each treatment was conducted in triplicate for 20 days. In the second experiment, the microalgae species from the best binary diet used in Experiment 1 (T-ISO and *C. muelleri*) were fed to *P. margaritifera* larvae individually at the same feeding rate used in Experiment 1 (Table 1). Each treatment was conducted in triplicate and the experiment was terminated after 9 days.

Student's t-test was used to determine whether the growth and survival of larvae differed significantly between diets in both experiments. Survival data (%) were arcsine transformed, prior to analysis.

Table 1. Rations of microalgae fed to *Pinctada margaritifera* larvae during Experiment 1 and Experiment 2.

Day	Feeding Rate (cell mL^{-1})
1	1,000
2	1,500
3-4	3,000
5	4,000
6	5,000
7	6,000
8	7,000
9	8,000
10	9,000
11-20	10,000

Results

Experiment 1

Growth of *P. margaritifera* larvae fed the two binary diets is shown in figure 1. At the end of the experiment, larvae fed T-ISO/*C. muelleri* had a mean (± SD) APM of 222.07 μm (± 21.94) and were significantly larger ($p < 0.05$) than those fed the T-ISO/*Pav. salina*, which had a mean (± SD) APM of 176.76 μm (± 16.09). There was no significant difference between

treatments on day 18; however, larvae fed T-ISO/*Pav. salina* showed little growth between day 18 and day 20 in contrast to those fed T-ISO/*C. muelleri*. A similar pattern was observed for larval survival, where larvae fed T-ISO/*C. muelleri* showed significantly higher ($P < 0.05$) survival (25 ± 4.8%) than those fed T-ISO/*Pav. salina* (7.1 ± 6.6 %) on day 20. A steep decline in survival for larvae fed both diets occurred between day 6 and day 10.

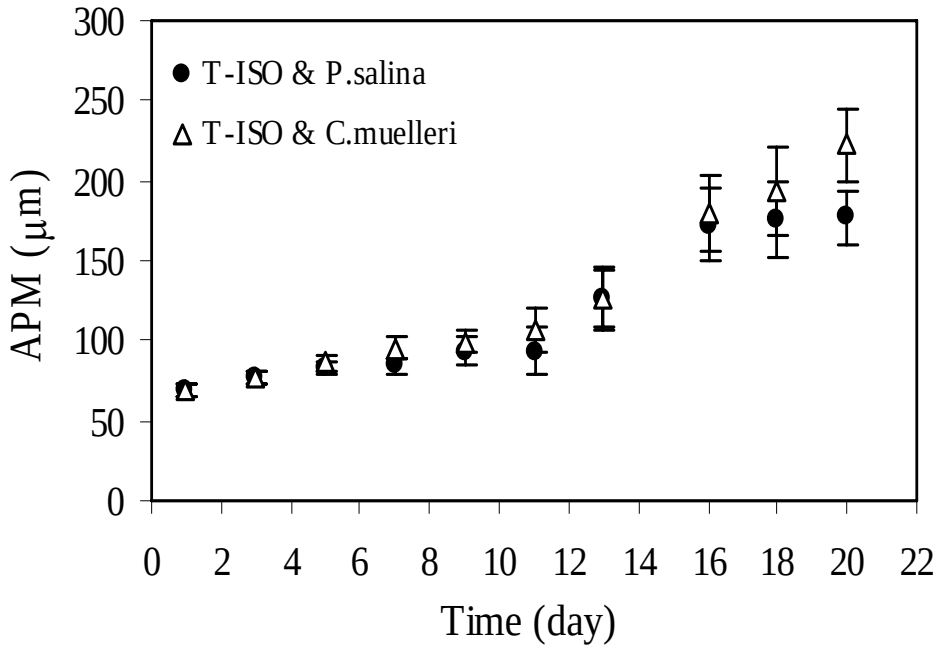

Fig. 1. Mean (± SD) change in antero-posterior measurement (APM) of *P. margaritifera* larvae fed two binary diets composed of either T-ISO/*Pav. salina* or T-ISO/*C. muelleri* in Experiment 1.

Experiment 2

The mean APM of larvae fed T-ISO at day 9 was 106 μm (± 8.1) and they were significantly larger ($P < 0.05$) than larvae fed *C. muelleri*, which had a mean APM of 93.3 μm (± 2.4) (Fig. 2). The larger size of larvae fed T-ISO was apparent starting from day 5 (Fig. 2). Larvae fed T-ISO recorded survival of 25.9% (± 9.1) at the end of the experiment, which was significantly higher ($P < 0.05$) than that of larvae fed *C. muelleri* (2.1 ± 2.7%).

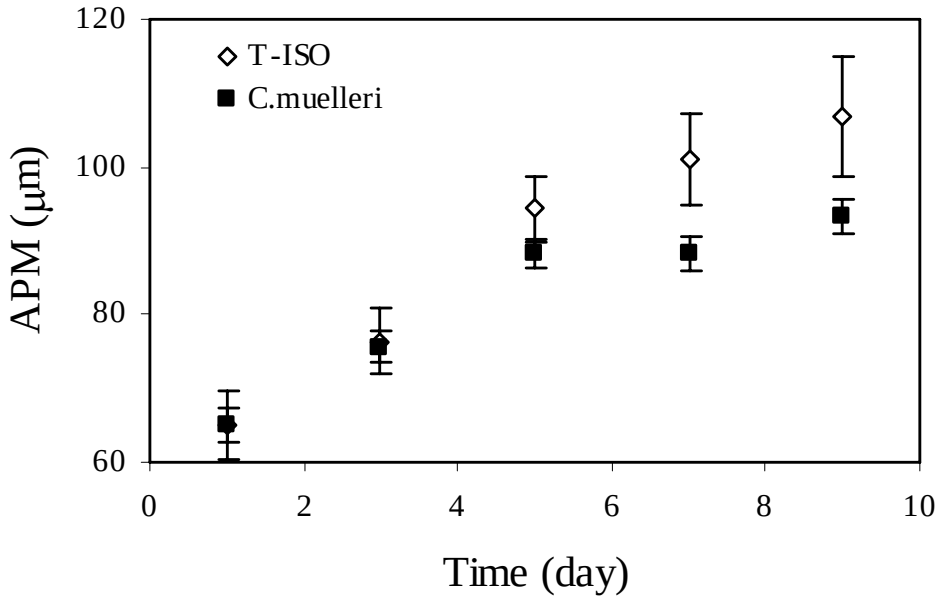

Fig. 2. Mean (± SD) change in antero-posterior measurement (APM) of *P. margaritifera* larvae fed either T-ISO or *C. muelleri* in Experiment 2.

Discussion

Although *P. margaritifera* larvae have been reared to settlement on different species of microalgae in previous studies (Tanaka 1970; Alagarswami 1989; Kakazu 1991; Southgate 1996; Southgate and Beer 1997; Southgate et al. 1998), this is the first study to report on the nutritional value of binary microalgal diets for *P. margaritifera* larvae. Southgate et al. (1998) assessed three species of tropical microalgae for *P. margaritifera* larvae (T-ISO, *Pav. salina* and *C. simplex*). They showed that T-ISO and *Pav. salina* supported good larval growth rates and suggested that a combination of these two species would provide a good diet for *P. margaritifera* larvae. The results of the present study indicate that a combination of T-ISO and *Pav. salina* does indeed support a good growth rate of *P. margaritifera* larvae; however, a combination of T-ISO and *C. muelleri* supported better larval growth than T-ISO/*Pav. salina*.

Although a combination of T-ISO and *C. muelleri* was a better dietary combination than T-ISO/*Pav. salina* for *P. margaritifera* larvae, the results of Experiment 2 showed that *C. muelleri* alone was a poor diet for

rearing *P. margaritifera* larvae up to 9 days old. Early *P. margaritifera* larvae fed *C. muelleri* had high mortality and poor growth. Species of *Chaetoceros* are generally considered to be of high nutritional value for bivalves (Enright et al. 1986; Helm and Laing 1987) and *C. muelleri,* in particular, has been rated as a high quality diet for oyster juveniles (Enright et al. 1986; O'Connor et al. 1992) and pearl oyster (*P. maxima*) spat (Taylor et al. 1997). It is likely that the nutritional value of *C. muelleri* depends to a great extent on the developmental stage of the grazer. *C. muelleri* has a relatively large cell size (including spines) and this may have influenced ingestion by young *P. margaritifera* larvae. Doroudi et al. (2003) showed that clearance rate and ingestion rate of microalgae increased with increasing age of *P. margaritifera* larvae, but at all larval sizes tested, *C. muelleri* was ingested at a considerably lower rate than flagellates (T.ISO, *Pav. lutheri* and *Pav. salina*). In a recent study that assessed the nutritional value of various tropical microalgae for *P. margaritifera* larvae, *Chaeteceros* spp. were shown to have reduced nutritional value for D-stage *P. margaritifera* larvae than for older umbone larvae (Martinez-Fernandez et al. 2006). These results indicate that effective utilization of *Chaeteceros* spp. may be dependent upon the age and size of *P. margaritifera* larvae. Based on the results of Experiment 2 and those of other studies, it is perhaps surprising that a combination of T-ISO and *C. muelleri* supported better growth of *P. margaritifera* larvae than T-ISO/*Pav. salina* in Experiment 1. Larvae in Experiment 2 clearly ingested *C. muelleri* when fed alone. However, the rate of ingestion of this species compared to that of the flagellates was not assessed.

Southgate et al. (1998) reported rapid growth rates of *P. margaritifera* larvae fed T-ISO alone. However, they also reported that T-ISO does not support development of *P. margaritifera* larvae through metamorphosis. This was assumed to result from shortcomings in the highly unsaturated fatty acid (HUFA) content of T-ISO (Southgate et al. 1998). The HUFA's eicosapentaenoic acid (20:5*n*-3; EPA) and docosahexaenoic acid (22:6*n*-3; DHA) are considered essential dietary components for bivalves (Langdon and Waldock 1981; Pilsbury 1985; Enright et al. 1986; Helm and Laing 1987). Although T-ISO contains high levels of DHA, EPA content is relatively low in this species (Volkman et al. 1989; Martinez-Fernandez et al. 2006). Conversely, *C. muelleri* contains relatively high levels of EPA, but DHA accounts for < 0.8% of total fatty acids in *Chaetoceros* species (Volkman et al. 1989). Our results indicate that the binary diet composed of T-ISO and *C. muelleri*, provided sufficient dietary HUFA's to support good growth of *P. margaritifera* larvae. Martinez-Fernandez et al. (2006)

reported that the growth of young (2-11 days old) *P. margaritifera* larvae was positively and significantly correlated with DHA content of dietary microalgae but not with EPA content. Furthermore, while growth was not significantly correlated with either EPA or DHA content of microalgae fed to older (11-19 days old) larvae, it was positively and significantly correlated with EPA+DHA content of microalgae fed to both young and older larvae (Martinez-Fernandez et al. 2006). Bivalves are usually fed more than one species of microalgae to provide a better 'balance' of nutrients and minimise dietary deficiencies in component species (Webb and Chu 1983).

The inferior growth of *P. margaritifera* larvae fed T-ISO/*Pav. salina* when compared to T-ISO/*C. muelleri* is interesting as the combination of these microalgae species was recommended for *P. margaritifera* larvae by Southgate et al. (1998) and an equal mixture of T-ISO and *Pav. salina* has been used routinely in other studies (Doroudi et al. 1999; Doroudi and Southgate 2000) with good results. Both T-ISO and *Pav. salina* have been shown to be of high nutritional value to *P. margaritifera* larvae when fed singly, with *Pav. salina* supporting particularly good growth rates (Martinez-Fernandez et al. 2006). All three microalgae used in this study are of tropical origin and considered suitable for use under tropical conditions (Jeffrey et al. 1992) such as those in Kiribati. Such species are tolerant to the higher water temperatures used in the culture of the larvae of tropical species. Prior research into the hatchery culture of tropical pearl oyster species has highlighted problems when temperate species of microalgae were used as a food source (Minaur 1969; Tanaka et al. 1970) and Minaur (1969) suggested that tropical microalgae were better suited for rearing the larvae of tropical pearl oysters. As more species of tropical microalgae species are isolated and become available for hatchery use, the options for component species used for rearing *P. margaritifera* larvae will increase as will the need for further research in this field.

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