Variation in clearance and ingestion rates by larvae of the black-lip pearl oyster (*Pinctada margaritifera*, L.) feeding on various microalgae

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Abstract

Clearance rate (CR) and ingestion rate (IR) of different sizes (89, 125 and 188 μm shell length) of *Pinctada margaritifera* larvae were determined when feeding on various microalgae. The microalgae tested were the diatoms, *Chaetoceros muelleri* and *C. simplex*, and flagellates, Tahitian *Isochrysis* aff. *galbana*, *Pavlova lutheri* and *P. salina* at 5 or 10 cells μL^{-1}. Both CR and IR of microalgae tested in this study increased with increasing larval size; but at all larval sizes, diatoms resulted in lower CR and IR. Of the microalgae tested, *P. margaritifera* larvae showed greatest CR and IR with the two *Pavlova* spp. Maximum CR for *P. salina* was 10.5, 21.2 and 29.7 $\mu L\ h^{-1}$ for larvae with shell lengths of 89, 125 and 188 μm, respectively. The highest IR values for *P. margaritifera* larvae with shell lengths of 89, 125 and 188 μm were 8.7, 81.0 and 165.7 cells·$larva^{-1}\ h^{-1}$, respectively. CR and IR of *P. salina* were approximately five times higher than those recorded for *C. muelleri* and *C. simplex*.

KEY WORDS: clearance, ingestion, larvae, microalgae, pearl oyster, *Pinctada margaritifera*

Received 2 January 2001, accepted 4 January 2002

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Introduction

Some research has suggested that temperate microalgae are unsuitable for rearing larvae of tropical pearl oysters because of the high water temperature at which the larvae are reared (Minaur 1969; Tanaka *et al.* 1970). Thus, Southgate *et al.* (1998) assessed the nutritional value of three species of tropical microalgae (Tahitian *Isochrysis* aff. *galbana*, *Pavlova salina* and *Chaetoceros simplex*) for larvae of *Pinctada margaritifera*. They reported significant differences in the nutritional value of the three species tested. The nutritional value of microalgae to bivalve larvae is influenced by many factors, including size, morphology and chemical composition. Particle capture by bivalve larvae depends on morphological characteristics of the larvae, such as length and velocity of the preoral cilia and the length of the velar edge (Strathmann & Leise 1979; Gallager 1988; Riisgard *et al.* 2000).

Larval nutrition has an important role in maximizing larval growth and survival in bivalve hatcheries; however, there is a paucity of information on the nutrition of pearl oyster larvae. Clearly, clearance rate (CR) and ingestion rate (IR) of microalgae will have a major influence on the growth of bivalve larvae and, on this basis, it is important that the microalgae chosen as larval foods are readily ingested. However, no prior study has reported on the CR and IR of pearl oyster larvae feeding on different species of microalgae and how these vary with larval age. Therefore, this study examined the relative CR and IR of different ages of *P. margaritifera* larvae feeding on various species of microalgae (flagellates and diatoms).

Materials and methods

Microalgae

Microalgae starter cultures were obtained from CSIRO Fisheries Division in Hobart, Tasmania, and the codes below refer to CSIRO catalogue codes. Algae were cultured in 3-L glass flasks in autoclaved 0.45-μm-filtered and UV-treated seawater using f/2 nutrient medium (Guillard 1983). Algae were fed to larvae during the exponential growth phase.

Larval rearing

Pinctada margaritifera larvae were reared in 500 L tanks according to the methods of Southgate & Beer (1997). They were fed a 1 : 1 mixture of Tahitian *Isochrysis* aff. *galbana* clone T-ISO (CS 177) and *P. salina* (CS 49) at a density of 1–20 cells μL^{-1}, based on previous studies with *P. margaritifera* larvae (Southgate & Beer 1997; Doroudi *et al*. 1999). Larvae were taken from these cultures for use in experiments. Larvae were unfed for 24 h prior to the start of experiments to maximize CR and IR (Sprung 1985; Gallager 1988).

Clearance and ingestion rates

Larvae were transferred to triplicate 500 mL beakers at three different ages and sizes, 5 days (89 ± 3.1 μm shell length, SL, $n = 30$), 10 days (125 ± 10.4 μm SL, $n = 30$) and 20 days (188 ± 11.8 μm SL, $n = 30$) after fertilization. Larvae were fed with one of five microalgae: T-ISO, *Pavlova lutheri, P. salina, Chaetoceros muelleri* and *C. simplex*, at a density of 5 or 10 cells μL^{-1}. The higher microalgae density (10 cells μL^{-1}) was only used for the older (188 μm SL) larvae. Experiments were conducted at 27 °C and 34% salinity. Sufficient larvae (20–30 mL^{-1}) were used in each experiment to achieve a marked reduction in algal density. A set of beakers with no larvae were used as controls to determine change in algal density independent of larval feeding. Samples (20 mL) were taken from each beaker at the start of the experiments and every 2 h thereafter, for a period of 6 h. After removing larvae from these samples using a 25 μm sieve, algal cells were counted using a Multisizer high speed particle counter Model II (Coulter Multisizer, Coulter, Fullerton, CA) fitted with a 70 μm aperture tube.

CR was calculated from the following formula, which was modified from Lucas (1982) to include a correction factor from the control:

$$\mathrm{CR}(\mu\mathrm{L\,larvae^{-1}\,h^{-1}}) = v[t^{-1}\ln(C_0/C_t) - a]$$

where v is the volume of water per larvae (μL), t the duration of experiment (h), C_0 the initial cell density (cells μL^{-1}), C_t the final cell density (cells μL^{-1}), and a the correction factor from control

$$\mathrm{a}(\mathrm{h^{-1}}) = t^{-1}\ln(C_0/C_t')$$

where C'_t is the final cell density in control (cells μL^{-1}).

Weight-specific CR_s (μL μg^{-1} L^{-1}) was calculated using the following equation (Lu & Blake 1997):

$$\mathrm{CR_s} = \mathrm{CR/AFDW}$$

where AFDW is the ash-free dry weight of larvae (μg).

IR at any mean cell concentration, was obtained by the equation:

$$\mathrm{IR(cells\,larvae^{-1}\,h^{-1})} = \mathrm{CR}(\mu\mathrm{L\,larvae^{-1}\,h^{-1}}) \times \bar{C}(\mathrm{cells}\,\mu\mathrm{L^{-1}})$$

where $\bar{C}$ is the mean cell concentration (cells μL^{-1})

$$= C_0 - C_t/t[(\mathrm{CR}/v) + a]$$

(Lucas 1982)

Measurements and data analysis

Shell length values of larvae were recorded from samples of 30 larvae using a graduated eyepiece in a microscope. AFDW values for larvae were obtained by collecting a known number of *P. margaritifera* larvae on a Whatman GF/C filter, drying them at 60 °C for 48 h and ashing them in a furnace at 450 °C for 4 h (Epifanio 1979). Each analysis was conducted three times.

Two-way analysis of variance (ANOVA) was used to test the effect of microalgae species, body size and interaction between them on CR and IR. Statistical analyses were performed using the SPSS computer software.

Results

Allometric relation

The relationship between AFDW and SL for *P. margaritifera* larvae is expressed by the following allometric equation and is shown in Fig. 1:

$$\mathrm{AFDW} = (1.5 \times 10^{-4})\mathrm{SL}^{1.515} (r = 0.91; n = 6)$$

As AFDW is related to volume, a three-dimensional unit (length^3), while SL is a measure of length, an exponent of about 3 could be anticipated. However, the exponent in this allometric relationship was only about 1.5. Thus, AFDW, a measure of organic content of the larvae, declined relative to SL with increasing size (age) of the larvae. Either AFDW decreased relative to size as the larvae grew or there was a change in dimensions of the larvae such that SL increased relative to body width and height, or both these changes occurred.

Clearance and ingestion rates

CR, IR and weight-specific CR_s for larvae of *P. margaritifera* offered T-ISO, *P. lutheri, P. salina, C. muelleri* and *C. simplex* separately for a period of 6 h are shown in Figs 2–4, respectively. Maximum CR for each size of larvae occurred

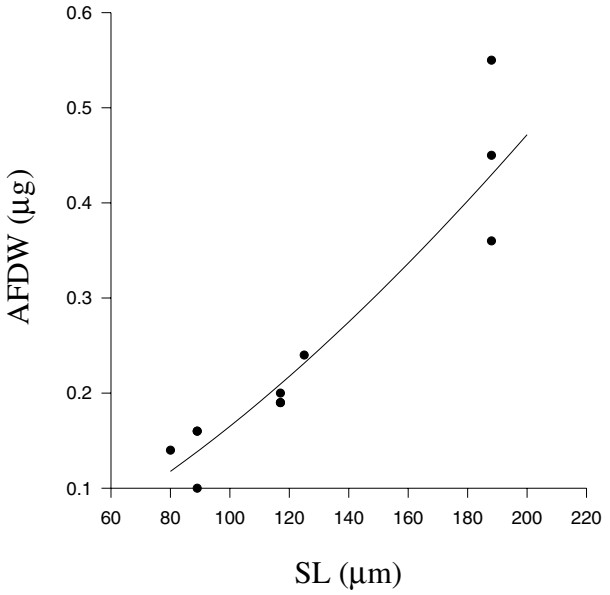

Figure 1 Allometric relationships between ash-free dry weight (AFDW, µg) and shell length (SL, µm) in *Pinctada margaritifera* larvae.

when fed *P. salina*; these were 10.5, 21.2 and 29.7 µL larvae^{-1} h^{-1} for larvae with SL of 89, 125 and 188 µm, respectively (Fig. 2). CR was next highest for larvae feeding on *P. lutheri* and then the other flagellate T-ISO. CR was lowest for the two diatoms (*Chaetoceros* spp.) at approximately 20–25% that of larvae feeding on *P. salina*. IR is related to CR and there was a similar pattern of high IR values for larvae feeding on *P. salina* with the lowest values for the diatoms (Fig. 3). The highest IR values for larvae of 89, 125 and 188 µm SL were 8.7, 81 and 165.7 cells larvae^{-1} h^{-1}, respectively. Both CR and IR increased with larval size with all species of microalgae tested. This effect was most pronounced for IR and specifically for IR values for larvae feeding on the *Pavlova* spp. (Fig. 3). There was, however, no clear pattern of changing CR_s with increasing larval size (Fig. 4).

Two-way ANOVA showed that CR and IR varied significantly according to microalgae species ($P < 0.01$) and larval size ($P < 0.01$). CR and IR were significantly effected by type of microalgae (flagellates vs. diatoms), whereas they were not significantly different between the two *Pavlova* spp. or between the two *Chaetoceros* spp. There was a significant interaction ($P < 0.01$) between the effects of both microalgae species and larval size on CR and IR.

Discussion

This study showed that, of the five microalgae assessed, *Pinctada margaritifera* larvae showed more rapid CR and IR of flagellates (*Pavlova* spp. and T-ISO) when compared with the two diatoms (*Chaetoceros* spp.) tested. This is likely to reflect differences in cell size or shape between the flagellates and diatoms tested. The microalgae assessed in this study were in the range of 4–6 and 6–8 µm for flagellates and diatoms, respectively (based on measure-

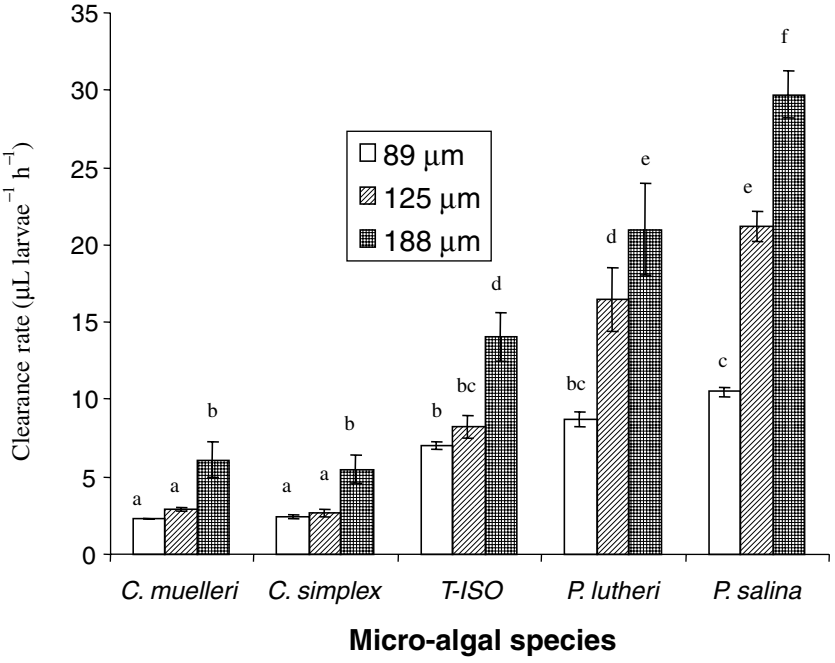

Figure 2 Clearance rates (µL larvae^{-1} h^{-1}) for three developmental sizes (89, 125 and 188 µm) of *Pinctada margaritifera* larvae feeding on various microalgae (*Isochrysis* T-ISO, *Pavlova lutheri, P. salina, Chaetoceros muelleri* and *C. simplex*).

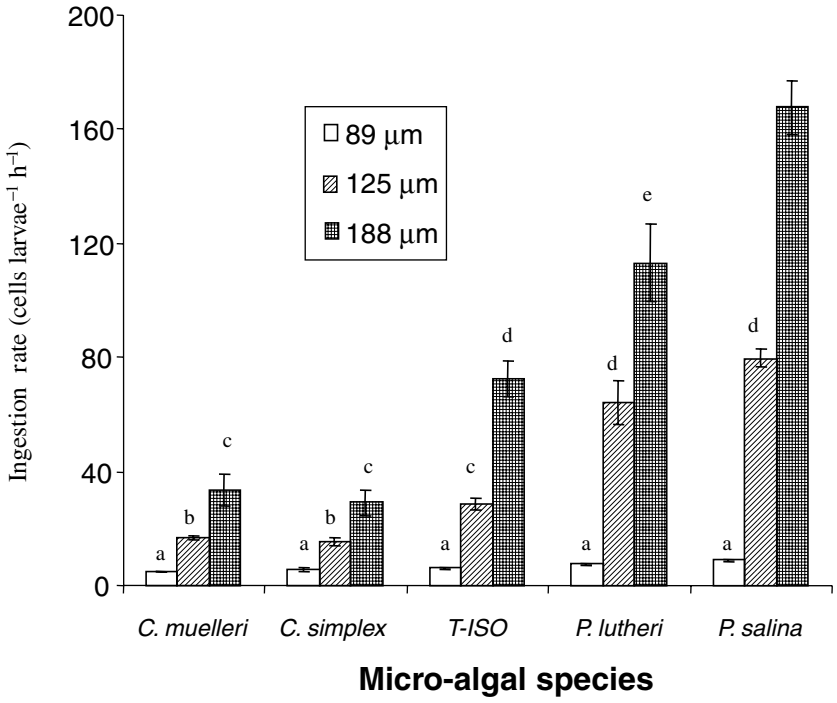

Figure 3 Ingestion rates (cells larvae^{-1} h^{-1}) for three developmental sizes (89, 125 and 188 µm) of *Pinctada margaritifera* larvae feeding on various microalgae (*Isochrysis* T-ISO, *Pavlova lutheri, P. salina, Chaetoceros muelleri* and *C. simplex*).

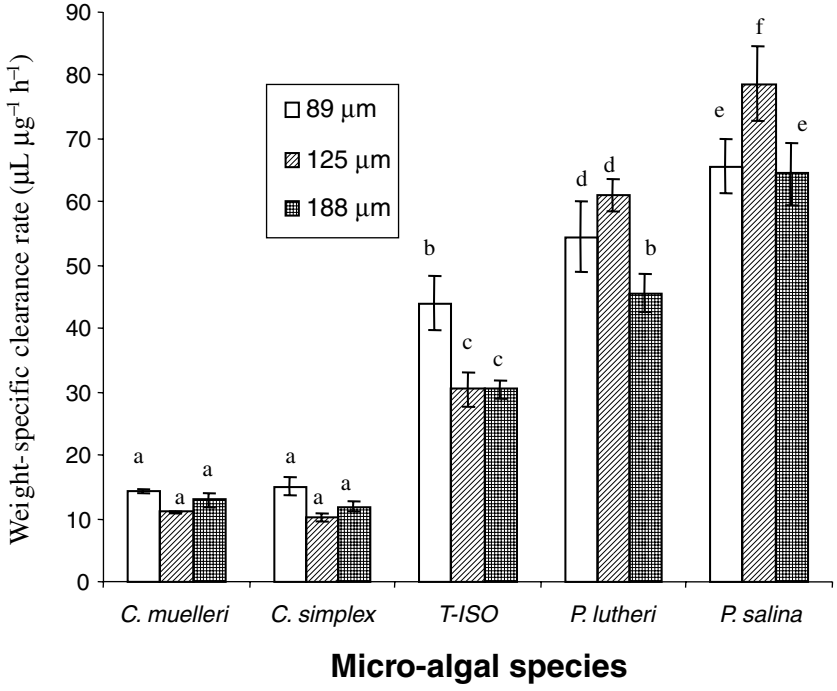

Figure 4 Weight specific clearance rates (mL µg^{-1} h^{-1}) for three developmental sizes (89, 125 and 188 mm) of *Pinctada margaritifera* larvae feeding on various microalgae (*Isochrysis* T-ISO, *Pavlova lutheri*, *P. salina*, *Chaetoceros muelleri* and *C. simplex*).

ment using a multisizer). Riisgard *et al*. (1980) and Sprung (1985) concluded that, within a range of 1–9 µm, *Mytilus edulis* larvae showed maximum CR of 3 µm particles. The major difference between flagellates and diatoms is that the latter has spines (Hoff & Snell 1987) which may make diatoms more difficult to capture and ingest using a ciliary system. Nevertheless, diatoms are widely used in diets for bivalve larvae (Brown *et al*. 1989) and information on their

relative CR and IR is important in developing more efficient diets.

Of the flagellates assessed in this study, *Pavlova* spp. showed significantly greater CR and IR than T-ISO. In a similar study with *Pteria sterna*, microscopic observations showed the gut to contain relatively more *P. lutheri* than *Isochrysis galbana* when larvae were fed equal mixture of both species (Araya-Nunez *et al.* 1995). The preferential ingestion of *Pavlova* in this study and the high CR and IR recorded in the present study may reflect morphological differences between *Pavlova* spp. and T-ISO or differences in the 'taste' of algae (O'Meley & Daintith 1992) reflecting differences in biochemical composition (Brown *et al.* 1997). Bayne *et al.* (1977) suggested that higher retention of *Phaeodactylum tricornutum* by *M. edulis* larvae may be a function of its cell shape and structure. Differences between the CR of *Pavlova* and T-ISO, which have a similar cell size, imply independent control over particle capture. Gallager (1988) reported that selection at the mouth in bivalve larvae, which could be passive or active, is a reason for differences in CR between *Synechococcus* and T-ISO by larvae of *Mercenaria mercenaria*.

CR of all species of microalgae tested increased with increasing larval size. Similarly, Wilson (1980) reported that CR increased with increasing size of *Ostera edulis* larvae. However, Gallager (1988) reported that rapid CR of smaller microalgae might be particularly significant for younger larvae where the diameter of the oesophagus and volume of the gut may limit the size and number of particles ingested. This might be expected, given that there is little change in the function of the filter-feeding system of bivalves larvae before metamorphosis to spat (Hickman & Gruffydd 1971) and the result of this study showed that larval size had no effect on microalgae preference during larval development.

The results of this study have shown that the CR and IR of *P. margaritifera* larvae feeding on the flagellates T-ISO and *P. salina* are high. Based on these results, and those of Southgate *et al.* (1998) who showed T-ISO and *P. salina* to be of high nutritional value for *P. margaritifera* larvae, a combination of T-ISO and *P. salina* is likely to be a suitable diet for *P. margaritifera* larvae. It should be noted that *P. lutheri* was ingested to a greater degree than T-ISO and at a similar rate to *P. salina*. However, previous research with pearl oyster larvae has shown that *P. lutheri*, a temperate species with high nutritional value (Jeffrey *et al.* 1990), is unsuitable for rearing larvae of tropical bivalves at relatively high water temperatures (Minaur 1969; Tanaka *et al.* 1970).

Acknowledgements

We thank Dr David Klumpp for facilities at the Australian Institute of Marine Science and for advice during this study. Mr Robert Mayer, Queensland Department of Primary Industries and Dr Sjaak Lemmens of Brown and Root Co. Pty. Ltd. provided helpful comments on the manuscript. This study was funded by the Australian Center for International Agricultural Research (ACIAR) as part of Project Number FIS/97/31 'Pearl Oyster Resource Development in the Pacific Islands'.

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