Available online at www.sciencedirect.com
SCIENCE DIRECT®
Aquaculture 230 (2004) 417–423
Aquaculture
www.elsevier.com/locate/aqua-online

Ingestion and digestion of 10 species of microalgae by winged pearl oyster *Pteria sterna* (Gould, 1851) larvae

Erika Martínez-Fernández[a,*], Héctor Acosta-Salmón[a,1], Carlos Rangel-Dávalos[a,b]

[a] *Laboratorio Experimental de Acuacultura, Unidad Pichilingue, Universidad Autónoma de Baja California Sur, A. P. 18-B, La Paz, B. C. S. 23081, Mexico*
[b] *Dirección General de Investigación en Acuacultura, Instituto Nacional de la Pesca, Mexico*

Received 27 March 2003; received in revised form 3 June 2003; accepted 3 June 2003

Abstract

Ten species of microalgae were tested for ingestion and digestion in *Pteria sterna* larvae using epifluorescence microscopy to choose an appropriate diet. An experiment was conducted using 2, 4, 5 (straight-hinge) 10 and 22 (umbo stage) day old larvae. Larvae were stocked in 150 ml flasks at 30 ml^{-1} and fed 100,000 algal cells ml^{-1} of each species individually. Larvae were fed for 1 h and then were observed under the microscope to detect ingestion; larvae were then sieved and placed in flasks containing filtered seawater and were observed after 1 and 2 h to analyse digestion for the microalgae ingested. Out of the 10 species administered, only *Nannochloris* sp., *Pavlova lutheri* and *Isochrysis* aff. *galbana* (T-ISO) were ingested, and only the last two species were digested. No ingestion of *Phaeodactylum tricornutum*, *Chaetoceros muelleri*, *Ch. calcitrans*, *Thalassiosira weisflogii*, *Dunaliella salina*, *Tetraselmis tetrathele* and *T. suecica* was evident at any stage of larval development tested. Only T-ISO and *P. lutheri* should be used for larval rearing of *P. sterna* until other species are identified as suitable (ingested and digested) for this species.

Keywords: Digestion; Epifluorescence microscopy; Ingestion; Larvae; Microalgae; *Pteria sterna*; Pearl oyster

* Corresponding author. Current address: Pearl Oyster Research Group, School of Marine Biology and Aquaculture, James Cook University, Townsville, Queensland 4811, Australia. Tel.: +61-7-47816323; fax: +61-7-47814585.
E-mail address: Erika.MartinezFernandez@jcu.edu.au (E. Martínez-Fernández).
[1] Current address: Pearl Oyster Research Group, School of Marine Biology and Aquaculture, James Cook University, Townsville, Queensland 4811, Australia.

0044-8486/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/S0044-8486(03)00416-2

1. Introduction

The pearl fishing effort from the 16th century up to the early 1900s that gave to the Baja California Peninsula and the "Sea of Cortez" its world-recognised fame as producers of pearls of excellent quality and beauty, also diminished the number of pearl oysters throughout the Mexican Pacific coast. Today both pearl oyster species, *Pteria sterna* (Gould) and *Pinctada mazatlanica* (Hanley), are enlisted as 'species under special protection' by the Mexican government.

Large variations in pearl oyster spat recruitment and the scarcity of wild stocks in the state of Baja California Sur limit the opportunity to develop a cultured pearl industry based only on wild spat collection (Martínez-Fernández et al., 2003). Hatchery-produced spat can support further development of the pearl industry in places with low spatfall in Mexico; however, it is necessary to improve current technologies to achieve the best yield from hatchery spat production and the nutritional aspects of larvae are of the utmost importance.

There have been some advances on pearl oyster larval nutrition in areas such as feeding protocols (Doroudi and Southgate, 2000; Doroudi et al., 1999) and microalgae replacements (Southgate et al., 1998), but much work is still needed to understand better the nutritional requirements of pearl oyster larvae. Not all microalgae species can be ingested or digested by small veliger larvae.

Cell size, digestibility and biochemical composition are factors determining the nutritive quality of the microalgae and their utility as food for bivalves, of these, digestibility can be one of the main factors determining larval growth and survival (Owen, 1974; Ewart and Epifanio, 1981; Albentosa et al., 1993). Results obtained during larval rearing of different bivalve species using some microalgal species may not be related to biochemical composition of the algae but to the inability of the larvae to ingest or digest such microalgae species.

Epifluorescence microscopy is effective in detecting the processes of ingestion and digestion of microalgae by bivalve larvae (Lucas and Rangel, 1983; Rangel-Davalos, 1984). Studies have shown that not all microalgae species are readily ingested and/or digested by bivalve larvae (Babinchak and Ukeles, 1979; Le Pennec and Rangel-Davalos, 1985; Lora-Vilchis and Maeda-Martínez, 1997); the fact that particular microalgae species are ingested does not necessarily mean they are digested. For example, out of 10 species of microalgae fed to *Argopecten ventricosus-circularis* (Sowerby II, 1842) larvae only 7 species were ingested and from those only 5 were digested (Lora-Vilchis and Maeda-Martínez, 1997).

Many microalgae species have been tried on pearl oyster larvae, however it is not known if they are either ingested or digested. The aim of this study was to identify which species of microalgae can be ingested and digested by the pearl oyster *P. sterna* larvae of different ages in order to design a larval feeding protocol.

2. Materials and methods

P. sterna broodstock used in this study were hatchery produced in 1997 and were maintained on a bottom culture system at El Merito, La Paz Bay, Baja California Sur,

Mexico. Oysters were transferred in February 2000 to the laboratory where they were cleaned prior to spawning. Spawning induction and larval rearing were conducted in winter using techniques described by Martínez-Fernández et al. (2003). This experiment was carried out in the UABCS's Aquaculture Experimental Laboratory of Pichilingue (LEAP) using a direct method (epifluorescence microscopy) to observe ingestion and digestion of microalgae species by *P. sterna* larvae. Larvae were fed a 1:1 mixture (by cell number) of *Isochrysis* aff. *galbana* (T-ISO) and *Pavlova lutheri* (PAV) at 10,000 cells ml^{-1} from day 2 up to day 18 and 15,000 cells ml^{-1} from day 20 until settlement (day 38).

2.1. Microalgae cultures

Microalgae (T-ISO and PAV) used to maintain the whole batch of larvae were produced in a semicontinuous (Fogg, 1995; Brown et al., 1993) system (immediately after harvesting, the culture volume was restored with new FSW and culture medium) in 100-l fiberglass tanks using Guillard F/2 medium, replacing cultures every 7 days. Microalgae used for the trials of ingestion and digestion were cultured in 500-ml Erlenmeyer flasks using Guillard F/2 medium. Microalgae were harvested during the exponential growth phase. Photoperiod was 24:0 (light/dark) and illumination was provided by 20-W cool white lamps irradiating 80 μmol m^{-2} s^{-1}. The algal species are shown in Table 1.

Table 1
Microalgae species used to feed *Pteria sterna* larvae

Group (Division)	Class[1]	Species[2]	Size (μm)	Code	Characteristics[3]
Diatoms (Bacillariophyta)	Bac	*Phaeodactylum tricornutum*[a] (Bohlin)	25 × 5	PHA	Elongated cells, large spines
	Cos	*Chaetoceros calcitrans*[b] (Paulsen) Takano	4–5	CHC	Rigid cell wall, large spines
		Ch. muelleri[b] (Lemmermann)	5 × 5	CHM	
		Thalassiosira weisflogii[a] (Grun.) F&H	11 × 4	THA	
Green algae (Chlorophyta)	Chl	*Dunaliella salina*[a] (Teodoresco)	8–10	DUN	Motile large cells, two flagella
		Nannochloris sp.[c]	2–3	NAN	Very small. Fibrous glycoprotein cell wall
	Pra	*Tetraselmis tetrathele*[d] (G.S. West)	8 × 16	TET	Motile large cells, four flagella. Cell wall covered of organic scales
		T. suecica[d] (Kylin)	12–15	TES	
Flagellates (Haptophyta)	Pry	*Isochrysis* aff. *galbana*[d] (Green)	6–8	T-ISO	Two flagella, round–oval shaped. Cell wall covered of polysaccharide scales
		Pavlova lutheri[d] (Droop)	5	PAV	

[1]Bac, Bacillariophyceae; Cos, Coscinodiscophyceae; Chl, Chlorophyceae; Pra, Prasinophyceae; Pry, Prymnesiophyceae.

[2a]Unknown origin; [b]CICESE; [c]CICIMAR; [d]CIBNOR.

[3]Borowitzka and Borowitzka (1988); Van den Hoek et al. (1995).

Table 2
Ingestion and digestion stages used in this study

Stage	Fluorescence	Characteristics
(I) Ingestion	Red	Whole algal cells well defined in the stomach
(II) Digestion	Tones of Pink, Orange or Yellow	Whole and lysed algal cells mixed in the stomach or no whole cells present (lysed algae only)
(III) Empty	No fluorescence	Empty stomach: larvae were not fed or had finished digestion

Modified from Le Pennec and Rangel-Davalos (1985).

2.2. Ingestion and digestion trials

The experiment was conducted on days 2, 4, 5 (straight-hinge larvae), 10 and 22 (umbo stage larvae) after fertilisation. With exception of day 2 when no food had been previously administered, larvae were left unfed for 24 h prior to the experiment to assure the digestive gland was empty before testing ingestion and digestion. Larvae were stocked at 20 ml^{-1} in duplicate 150 ml flasks containing 100,000 algal cells ml^{-1}. Each microalgal species was administered individually. Larvae were allowed to feed for 1 h and were observed under a Nikon OPTIPHOT-2 epifluorescence microscope following Le Pennec and Rangel-Davalos (1985) to detect ingestion, then, they were sieved through a 47-μm mesh sieve, washed with FSW and placed in flasks containing new FSW to evaluate digestion. A group of unfed larvae was used as a control.

Samples of at least 30 larvae from each flask were observed in the epifluorescence microscope 1 and 2 hours after washing to identify digestion for the microalgae species ingested only. To determine the stages of ingestion and digestion, a modification of the scale proposed by Le Pennec and Rangel-Davalos (1985) was used; stages and characteristics are explained in Table 2. For practical reasons, differentiation of early and late digestion was not measured as inaccuracies reading the different colours (tones of pink, orange or yellow) with the naked eye can lead to confusion. Ingestion was defined as the stage when the larvae showed well-defined fluorescence inside the digestive gland, in this stage whole microalgae cells can be observed fluorescing individually. Digestion was defined as the stage when microalgal cells are not evident but fluorescence is still observed as a spot filling up the digestive gland.

3. Results

Larvae ingested only three species of microalgae: NAN, PAV and T-ISO (Table 3). Larvae ingested *Nannochloris* sp. (NAN), but were unable to digest it. For days 2 and 4, after 8 h from ingestion, no broken cells were observed. For days 5, 10 and 22, once the fed larvae were placed in filtered seawater, some cells were presumably expelled as the number of cells in the digestive gland was reduced without any evidence of digestion after 24 h.

Larvae ingested and digested PAV better than T-ISO in quantity and time. For all ages tested, larvae-fed PAV reached stage III within the 2-h period after washing; by this time,

Table 3
Stages of ingestion and digestion of *Nannochloris* sp. (NAN), Pavlova *lutheri* (PAV) and *Isochrysis* aff. *galbana* (T-ISO), after 1 and 2 h of placing *Pteria sterna* larvae in filtered seawater

Larval age (days)	2		4		5		10		22	
Time (h)	1	2	1	2	1	2	1	2	1	2
NAN	I	I	I	I	I	I	I	I	I	I
PAV	I	III	II	III	II	III	II	III	II	III
T-ISO	Not ingested		II	II	II	II	II	II	II	II

(I) Ingestion. (II) Digestion. (III) Empty stomach.

larvae-fed T-ISO showed some remaining fluorescence indicating digestion was still in progress, with the exception of 2-day-old larvae, where no ingestion of this species took place. The ingestion and digestion pattern was the same for all larval ages tested.

4. Discussion

Out of the 10 species of microalgae tested, only 2 species were digested. The remaining 8 species are thus unsuitable for feeding *P. sterna* larvae younger than 22 days, therefore they must be avoided during this period to avoid creating water quality problems when these species are not being consumed by the larvae.

Nannochloris species are readily ingested probably because of their small size ($\sim 2-3$ μm), however they are not easy to digest. In agreement with our results, *A. ventricosus-circularis* larvae ingested *Nannochloris oculata* cells, however no digestion was noticed (Lora-Vilchis and Maeda-Martínez, 1997).

We obtained similar results for ingestion and digestion of *Isochrysis* and *Pavlova* species to those observed for other bivalve species such as *Crassostrea virginica* (Babinchak and Ukeles, 1979), *Pecten maximus* (Le Pennec and Rangel-Davalos, 1985) and *A. ventricosus-circularis* (Lora-Vilchis and Maeda-Martínez, 1997). These two algal species have been used to great extent for pearl oyster larval rearing including *P. sterna* (Araya-Nuñez et al., 1995; Rose and Baker, 1994; Doroudi and Southgate, 2000; Martínez-Fernández et al., 2003).

For the other microalgal species, problems related to the physical dimensions (form and size) of the cells were probably the cause that impeded ingestion by *P. sterna* larvae. Large round cells like *Tetraselmis tetrathele* (TET), *T. suecica* (TES) and *Dunaliella salina* (DUN) (of up to 15 μm in diameter) may be too large for the larvae to ingest, large spines on the diatoms (CHC, CHM, THA and PHA) may have hampered ingestion as reported by Rose and Baker (1994) and Lora-Vilchis and Maeda-Martínez (1997).

Some *Chaetoceros* species have been used as larval rearing diet for some pearl oyster species (Rose and Baker, 1994; Southgate and Beer, 1997; Doroudi et al., 2003) and also have been proved to be very valuable in enhancing the nutritional value of mixed larval rearing diets (Brown and Robert, 2002). However, our study shows that *P. sterna* did not

ingest the *Ch. calcitrans* (CHC) and *Ch. muelleri* (CHM) species during most of its larval stage.

Epifluorescence technique demonstrated the ineffectiveness in the use of 8 of the 10 microalgae species to feed D-shape and umbo stage larvae of *P. sterna*; however, since our work was done with larvae younger than 22 days, it would be advisable to test these species in older larvae, since age may be an important factor in digestive performance. Similarly, it would also be advisable to isolate and test small native microalgae (specially diatoms) available for this species in its natural environment. In the mean time, *P. sterna* larvae younger than 22 days should be fed only on PAV and T-ISO and other species should be avoided until further evaluation of ingestion and digestion indexes.

We recommend to test ingestion and digestion of the other eight microalgal species after larval settlement to identify if some of these can be administered as a nursery culture diet and improve the current technique. Other studies as testing different microalgae and larval densities, physical and chemical parameters and larval culture system (Doroudi et al., 1999; Doroudi and Southgate, 2000) should be addressed, to achieve the best yield on the hatchery spat production this species.

Acknowledgements

The authors thank the UABCS Marine Biology students who participated during the larval-rearing process and the ingestion and digestion experiment. We are grateful to Dr. Laura Castell (James Cook University, Australia) and Prof. Marcel Le Pennec (Institut Universitaire Européen de la Mer, France) for their critical review and comments on the manuscript.

References

Albentosa, M., Perez-Camacho, A., Labarta, U., Beiras, R., Fernández-Reiriz, M.J., 1993. Nutritional value of algal diets to clam spat *Venerupis pullastra*. Mar. Ecol., Prog. Ser. 97, 261–269.

Araya-Nuñez, O., Ganning, B., Buckle-Ramirez, F., 1995. Embryonic development, larval culture and settling of American pearl-oyster (*Pteria sterna*, Gould) spat. Calif. Fish Game 81, 10–21.

Babinchak, J., Ukeles, R., 1979. Epifluorescence microscopy, a technique for the study of feeding in *Crassostrea virginica* veliger larvae. Mar. Biol. 51, 69–76.

Borowitzka, M.A., Borowitzka, L.J., 1988. Micro-algal Biotechnology. Cambridge Univ. Press, Cambridge, Great Britain. 477 pp.

Brown, M., Robert, R., 2002. Preparation and assessment of microalgal concentrates as feeds for larval and juvenile Pacific oyster (*Crassostrea gigas*). Aquaculture 207, 289–309.

Brown, M.R., Garland, C.D., Jeffrey, S.W., Jameson, I.D., Leroi, J.M., 1993. The gross and amino acid compositions of batch and semi-continuous cultures of *Isochrysis* sp. (clone T.ISO), *Pavlova lutheri* and *Nannochloropsis oculata*. J. Appl. Phycol. 5, 285–296.

Doroudi, M.S., Southgate, P.C., 2000. The influence of algal ration and larval density on growth and survival of blacklip pearl oyster *Pinctada margaritifera* (L.) larvae. Aquac. Res. 31, 621–626.

Doroudi, M.S., Southgate, P.C., Mayer, J., 1999. Growth and survival of black lip pearl oyster larvae fed different densities of microalgae. Aquac. Int. 7, 179–187.

Doroudi, M.S., Southgate, P.C., Lucas, J.S., 2003. Variation in clearance and ingestion rates by larvae of the black-lip pearl oyster (*Pinctada margaritifera*, L.) feeding on various microalgae. Aquac. Nutr. 9, 11–16.

Ewart, J.W., Epifanio, C.E., 1981. A tropical flagellate food for larval and juvenile oysters, *Crassostrea virginica* Gmelin. Aquaculture 22, 297–300.

Fogg, G.E., 1995. Algal Cultures and Phytoplancton Ecology. University of Wisconsin Press, Milwaukee, USA. 126 pp.

Le Pennec, M., Rangel-Davalos, C., 1985. Observations en microscope a épifluorescence de l'ingestion et de la digestion d'algues unicellulaires chez des jeunes larves de *Pecten maximus* (Pectinidae, Bivalvia). Aquaculture 47, 39–51.

Lora-Vilchis, M.C., Maeda-Martínez, A.N., 1997. Ingestion and digestion index of catarina scallop *Argopecten ventricosus-circularis*, Sowerby II, 1842, veliger larvae with ten microalgae species. Aquac. Res. 28, 905–910.

Lucas, A., Rangel, C., 1983. Detection of the first larval feeding in *Crassostrea gigas*, using the epifluorescence microscope. Aquaculture 30, 369–374.

Martínez-Fernández, E., Acosta-Salmón, H., Rangel-Dávalos, C., Olivera, A., Ruiz-Rubio, H., Romo-Piñera, A.K., 2003. Spawning and larval culture of the pearl oyster *Pinctada mazatlanica* in the laboratory. World Aquac. 34, 36–39.

Owen, G., 1974. Feeding and digestion in the bivalvia. In: Lowenstein, O. (Ed.), Advances in Comparative Physiology and Biochemistry, vol. 5. Academic Press, New York, USA, pp. 1–35.

Rangel-Davalos, C., 1984. Contribution a l'étude de l'ingestion et de la digestion d'algues monocellulaires chez les larves veligers des bivalves. These de Doctorat de Spécialité, Université de Bretagne Occidentale, France. 134 pp.

Rose, R.A., Baker, S.B., 1994. Larval and spat culture of the Western Australian silver- or goldlip pearl oyster, *Pinctada maxima* Jameson (Mollusca: Pteriidae). Aquaculture 126, 35–50.

Southgate, P.C., Beer, A., 1997. Hatchery and early nursery culture of the black lip pearl oyster (*Pinctada margaritifera* L.). J. Shellfish Res. 16, 561–567.

Southgate, P.C., Beer, A., Duncan, P.F., Tamburri, R., 1998. Assessment of nutritional value of three species of tropical microalgae, dried *Tetraselmis* and a yeast-based diet for larvae of blacklip pearl oyster, *Pinctada margaritifera* (L.). Aquaculture 162, 247–257.

Van den Hoek, C., Mann, D.G., Jahns, H.M., 1995. Algae: An Introduction to Phycology. Cambridge Univ. Press, Cambridge, Great Britain. 623 pp.