Aquaculture 284 (2008) 98–105

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Combined effects of diet and stocking density on growth and biochemical composition of spat of the Cortez oyster *Crassostrea corteziensis* at the hatchery

José M. Mazón-Suástegui, Karen M. Ruíz-Ruíz, Aurora Parres-Haro, Pedro E. Saucedo *

Centro de Investigaciones Biológicas del Noroeste (CIBNOR), La Paz, B.C.S., Mexico

ARTICLE INFO

Article history:
Received 22 April 2008
Received in revised form 14 July 2008
Accepted 15 July 2008

Keywords:
Biochemical composition
Artificial-diet
Density
Hatchery
Crassostrea corteziensis
Spat

ABSTRACT

The interactive effects of diet and stocking density on growth and biochemical composition of hatchery-reared *Crassostrea corteziensis* spat were investigated. Specimens were maintained for 21 days in upwelling chambers with continuous flow-through of seawater containing feed. The diets were: (1) A 1:1 combination of the microalgae *Isochrysis galbana* and *Chaetoceros mulleri*, (2) A 50/50 mixture of the two microalgae and cornstarch, and (3) A 50/50 mixture of the two microalgae and wheat flour. Experimental densities of specimens per upwelling cylinder were: low (5714), medium (11,428), and high (17,142). Changes in growth of spat (shell height, total wet weight, and total volume) and biochemical composition of the meat (carbohydrates, proteins, and lipids) were measured. The diet of microalgae (firstly) and microalgae with cornstarch (secondly) led to faster growth of spat under low stocking density conditions. In contrast, spat grew significantly less in shell height, wet weight, and total volume at medium and high density when fed the microalgae/wheat flour diet. Highest protein, carbohydrate, and lipid content occurred with the diet containing only microalgae, regardless of density. Glycogen content did not vary significantly between diets 1 and 2. Our results confirm that microalgae continue to be the main food source for meeting nutritional needs of *C. corteziensis* spat. However, it is feasible to replace microalgae by < 50% with some smaller proportion of cornstarch without significantly affecting the nutritional balance of the diet or the biochemical composition of spat.

1. Introduction

Historically, bivalve hatcheries have relied on the culture of one or more species of microalgae as the main food source of larvae, spat, and adults (Brown et al., 1998; Knauer and Southgate 1999; Volkman and Brown, 2005). Although more than 100 species of microalgae have been tested as live feed for bivalves, some golden-brown flagellates (*Isochrysis* spp., *Pavlova* spp.) and diatoms (*Chaetoceros* spp.) are particularly relevant for bivalve aquaculture, given their nutritional profiles (Brown et al., 1998; Volkman and Brown, 2005) and excellent results in rearing bivalve larvae or spat (Martínez-Fernández et al., 2006; Rivero-Rodríguez et al., 2007).

Despite their value, live microalgae are expensive to produce in indoor facilities and may account for 30 to 50% of hatchery operating costs (Jeffrey and Garland, 1987; Coutteau and Sorgeloos, 1993). Therefore, during the last few decades, there has been a growing interest in diets made of low-cost nutritional feeds that substitute for part the biomass of live microalgae (Castell and Trider, 1974; Urban and Langdon, 1984). A wide variety of materials have been investigated, including dried macroalgae and microalgae, bacteria and yeasts, microparticulated food particles, cereal flours, etc. (see review by Knauer and Southgate, 1999). While most of these products have given satisfactory results in rearing marine bivalves, only a few have allowed replacing a large part of the algal proportion without affecting the nutritional balance of the diet or the condition of the animals. Cereals such as rice, oats, wheat, and corn are inexpensive, easy to prepare and assimilate, and energetically rich, and hence, have emerged as promising diets for meeting the nutritional needs of bivalves (Chanley and Normandin, 1967; Kuwatani and Niishi, 1968; Turgeon and Haven, 1978; Mazón-Suástegui, 1988; Mazón-Suástegui and Avilés-Quevedo, 1988; Fernández-Reiriz et al., 1998; Pérez-Camacho et al., 1998).

Additionally, stocking density is recognized as an important factor influencing intra-species competition for space and food of most marine invertebrates raised under hatchery or field conditions. In bivalves, the set density affects survival and growth, reproductive effort, sex ratio, and physiological status of the organisms (Hernández-Llamas, 1997; Taylor et al., 1997a; Mazón-Suástegui, 2005; Monteforte et al., 2005; Ruíz-García, 2006). Previously, the interactive effects of diet and stocking density on biological regulation of marine bivalves have not been documented.

The Cortez oyster *C. corteziensis* (Hertlein, 1951) inhabits tropical and subtropical coasts of the Pacific Ocean from Mexico to Peru (Stuardo and Martínez, 1975). Along the northwest coast of the

* Corresponding author. Centro de Investigaciones Biológicas del Noroeste (CIBNOR), Mar Bermejo 195, Col. Playa Palo de Santa Rita, La Paz, B.C.S. 23090, Mexico. Tel.: +52 612 123 8464; fax: +52 612 125 3625.
E-mail address: psaucedo04@cibnor.mx (P.E. Saucedo).

0044-8486/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2008.07.022

mainland of Mexico (Gulf of California), the species has received growing interest for exploitation in aquaculture because its meat has excellent flavor and increasing acceptance in regional markets, but also because the exotic oyster *C. gigas* commercially raised in this area has been suffering large and unexpected die-offs over the last decade (Vázquez-Juárez et al., 2006). Unfortunately, *C. corteziensis* is an overexploited species and its current fishery does not provide the numbers of oysters demanded by local markets. Therefore, scientific improvement of hatchery and field cultivation methods is crucial. At present, scientific knowledge of the species is limited, but includes basic studies of biochemical composition of tissues (Páez-Osuna et al., 1993), reproductive cycle (Frías-Espericueta et al., 1997), field cultivation (Chávez-Villalba et al., 2005), physiological regulation related to temperature (Cáceres-Puig et al., 2007), density effects during field cultivation (Ruíz-García, 2006), influence of nursery diets on grow-out to adult size (Leyva-Miranda, 2005), and nutritional requirements of spat (Rivero-Rodríguez et al., 2007; Ojeda-Ramírez et al., 2008).

To increase our knowledge of nutritional needs of *C. corteziensis* spat, this paper evaluated the response of specimens kept at the hatchery under different diets (natural vs. artificial) and stocking densities to define a realistic strategy for maximizing growth and condition of spat for field cultivation. The study forms part of broader research aimed to optimize hatchery culturing protocols of this species in Mexico.

2. Materials and methods

2.1. Origin of spat and experimental design

Spat used in this study were produced at the hatchery following methods developed by Mazón-Suástegui et al. (2002a). After a 3-week post-setting period, nearly 412,000 spat (2.56 ± 0.21 mm shell height and 0.028 ± 0.00016 g wet weight) were subjected to one of nine treatments consisting of a combination of three diets and three stocking densities. The diets were: (1) A 1:1 blend of the microalgae *Isochrysis galbana* and *Chaetoceros mulleri* as the control group; (2) A mix of the two microalgae with 50% of their biomass replaced with cornstarch, and (3) A mix of the two microalgae with 50% of their biomass replaced with wheat flour. The densities were defined by the number of spat raised in a culturing device: (1) Low (5,714), (2) Medium (11,428), and (3) High (17,142). These numbers were determined by volumetric displacement.

Experimental animals were raised in re-circulating upwelling chambers, designed as illustrated in Fig. 1 An upwelling chamber consisted of a 1000-L, fiberglass, circular tank provided with a bordering collecting channel, two PVC aero-siphons, and several 3-inch PVC couplings that held twelve, upwelling cylinders (60 cm height × 30 cm diameter). A variable number of aero-siphons helped generating a continuous and re-circulating flow of seawater that provided food to the spat beds, which were supported within each cylinder by a 500-μm, false-bottom plastic mesh. Each upwelling chamber received 2200–2800 L d^{-1} of fresh, filtered (1-μm) seawater (25 ± 1 °C, salinity of 37 ± 1). The chamber's design allowed seawater to be fully renewed 2.5 times d^{-1}. Each upwelling cylinder received 590–750 L h^{-1} of food-laden seawater, which was renewed 600–620 times d^{-1}. Three chambers were used for dietary treatments (one per diet); four cylinders were used for each density treatment within each chamber (total of 36 experimental units for all diet/density combinations) (Fig. 1). Cylinders were rotated daily to the next chamber's supporting coupling to provide homogeneous experimental conditions. To prevent bacterial infections, upwelling chambers were drained daily and the spat washed thoroughly with seawater first and then with tap water.

2.2. Food preparation

Stocks of algae were cultivated in 40-L plastic bags containing sterilized, filtered (0.5 μm) seawater, enriched either with f2 medium for *I. galbana* or f2+silicate solution for *C. muelleri* (Guillard, 1974). Cultures were grown at 23 ± 1 °C, a salinity of 37 ± 1, and a photoperiod of 12 h light to 12 h dark. As a routine daily procedure, cell density was determined with a particle counter (Beckman–Coulter MultiSizer 3, Fullerton, CA) to harvest each microalga in its exponential growth phase. After harvest, microalgae were mixed at a 1:1 ratio and immediately diluted to 300 cells μL^{-1} in two 5000-L fiberglass conical tanks containing filtered seawater and serving as reservoirs (Mazón-Suástegui, 2005). This mixture was continuously provided by gravity flow from the reservoir tanks to the nursery chambers.

Cereal diets were prepared by suspending commercial cornstarch (Maizena®, Unilever de México, Mexico City,) or finely grounded wheat flour (Harinera Hasaya, Mexico City) in 0.5 L cold drinking water. The resulting mixture was poured into 5-L boiling tap water and then gently stirred for 5 min. to ensure a complete and homogeneous cooking. Cereal powders were supplied at a ratio equivalent to 1.5% wet weight of spat. Dietary ration was adjusted every week proportionally to the increase in wet weight of the spat. Once prepared, cereal emulsions were diluted in two 500-L fiberglass, conical tanks (one per diet) containing 1-μm filtered seawater (25 ± 1 °C, salinity of 37 ± 1) with vigorous aeration to maintain the particles in suspension. Cereal emulsions were continuously supplied by gravity flow to the corresponding nursery chamber.

2.3. Absolute growth and growth rate

At the beginning of the trials, mean shell height, total wet weight, and total volume of spat were determined from the initial stock population. Measurements, however, were taken differently. Initial shell

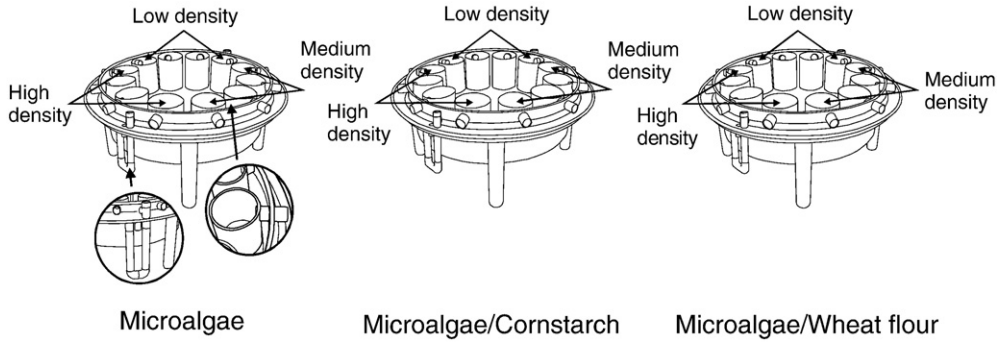

Fig. 1. Detailed scheme of the three upwelling chambers used for rearing spat of the Cortez oyster *Crassostrea corteziensis* under different combinations of diet and stocking density at the hatchery. Original source: Mazón-Suástegui (2005).