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Research Article

Cornstarch as a dietary supplement in conditioning broodstock and spat at a nursery of the Pacific calico scallop, *Argopecten ventricosus*

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ABSTRACT. Microalgae diets supplemented with cornstarch at a rate of 0.15% of live weight to the conditioning of broodstock and spat growth of Pacific calico scallop *Argopecten ventricosus* were tested. To determine reproductive condition at the beginning and end of the experiment, the gonads of adults were analyzed by stereological methods. For juveniles, the weekly percent increase of spat was estimated from spat biomass volume. The microalgae diet of *Tisochrysis lutea* and *Chaetoceros calcitrans*, supplemented with cornstarch, substantially improved the reproductive status of the scallop, particularly by increasing the number of individuals in mature and mature-spawning stages with more mature oocytes by the scallop. Spat growth improved >80% in the nursery with the diet supplemented with cornstarch compared with the microalgae diet (*T. lutea*, *C. gracilis*, and *C. calcitrans*). It can be suggested that a small addition of cornstarch to a microalgae diets, boosts growth during nursery procedures and performance of *A. ventricosus* during the routine conditioning of the broodstock.

Keywords: Pectinidae, nutrition, growth, gonadic condition, reproduction, aquaculture.

INTRODUCTION

Production of spat of bivalve mollusks under laboratory conditions starts with the gonadic conditioning of broodstock. Mollusks must reach sexual maturity and respond properly to spawn in a programmed way after receiving induction stimuli. It is expected that broodstock produces viable gametes and healthy larvae that develop and massively overtake the different larval stages and critical periods of metamorphosis and post-metamorphosis. If this is achieved, juveniles can be used as “seeds” in culture systems for grow-out in protected sea water. Besides controlling physical and chemical conditions, the diet is fundamental and should be based on microalgae with appropriate nutritional composition (Farías, 2001; Helm *et al.*, 2006; Goseling, 2015).

Bivalves feed by filtration and have the capacity of ingesting both live (particularly microalgae) and inert particles (organic and inorganic) in seston (Lucas, 1982). Rearing microalgae represent 30-50% of production costs of mollusk spat (Jeffrey & Garland, 1987; Coutteau & Sorgeloos, 1992). Hence, the search for diets with natural processed products (artificial feeds) is a relevant research topic to optimize cultivation of bivalve mollusks and reduce or replace live feed (microalgae).

Several studies have tested artificial formulations with potential use as dietary substitutes or supplements to live microalgae. Some involve microencapsulates that incorporate refined vegetable proteins, fish oil, and finely pulverized vegetables, grains and cereals with a high content of carbohydrates (Langdon & Siegfried,

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1984; Perez-Camacho *et al.*, 1998; Nevejan *et al.*, 2008). Diets based on yeasts (Coutteau *et al.*, 1994) and other raw materials have been tested. None of these alternative diets provides a complete substitution for live microalgae. Some formulations can be important supplements to the microalgal diet (Mamat & Alfaro, 2014). Research can find supplements that benefit the different stages toward the production of juveniles and do not restrict production logistics. This means that the use of these supplements should be cost-effective, particularly in the early stages with high food demand, like the conditioning of broodstock and nursery management of postlarval stages under controlled conditions. This is fundamental for raising juveniles to a size that can be manipulated, sown, and grown in the sea.

Cornflour or cornstarch is not a dietary substitute for microalgae because it is almost entirely carbohydrates. However, these flours are inexpensive, widely available, and easy to prepare as suspensions for filter feeders. These suspensions can be integrated with live diets, even used as a partial substitute of cultivated microalgae in adult and juvenile bivalves (Pérez-Camacho *et al.*, 1998; Albentosa *et al.*, 2002; Mazón-Suástegui *et al.*, 2008, 2009; Mamat & Alfaro, 2014).

The Pacific calico scallop *Argopecten ventricosus* (Sowerby, 1842) is a functional hermaphrodite bivalve, ranging from Santa Barbara, California, through the Gulf of California, to Bayovar, Piura in Perú (Coan & Valentich-Scott, 2012). It is under serious fishing pressure in northwestern Mexico, especially in the southern Baja California Peninsula. Natural banks have collapsed and this has led to government policies to increase production through aquaculture activities. Spat production technology in the laboratory has been successful, but optimization is necessary, to increase profits from grow-out until reaching commercial harvest size (Ruiz-Verdugo *et al.,* 2016).

Preliminary studies on the substitution of microalgae with artificial ingredients demonstrate the impossibility to completely eliminate microalgae, and suggest using cornstarch as a supplement for gonadal maturation of scallop broodstock (Mazón-Suástegui, 1988) and spat production in the nursery (Mazón-Suástegui & Avilés-Quevedo, 1988).

The objective of this study was to determine whether cornstarch supplementation enriches the natural diet (microalgae) of *A. ventricosus*, improving the gonadic conditioning process to obtain sexually mature broodstock and also, to enhance the spat growth during their nursery management at the hatchery.

MATERIALS AND METHODS

Diets and microalgal culture conditions

We tested a microalgal diet with a cornstarch supplement diet (microalgae+cornstarch) for conditioning of broodstock. The species tested were *Tisochrysis lutea*, previously named as *Isochrysis galbana* clone T-ISO (Bendif *et al*., 2013), and *Chaetoceros calcitrans.* The basic biochemical composition of the microalgae and cornstarch supplement diet used in this work is presented in Table 1.

Microalgae were cultivated in 40 L plastic bags, with 0.5 µm filtered seawater, sterilized by chlorination, and enriched with an f2 medium for the flagellate *T. lutea* and f2+silicate solution for the diatoms *C. calcitrans* and *C. gracilis* (Guillard, 1974). The cultures were maintained at 22 ± 0.5°C, 37.5 ± 0.5 salinity, and 24 h illumination. Cell density was recorded daily in each culture, using a particle counter (MultiSizer 3, Beckman-Coulter, Brea, CA) and harvesting each microalgal species in its exponential growth phase. After the daily harvest, the microalgae were mixed in a proportion of 1:1 for broodstock conditioning or 1:1:1 for spat grow-out and diluted with filtered sea water in 5000 L tanks. Food mix was provided by gravity to the experimental units of broodstock tanks and spat up dwellers (Mazón-Suástegui *et al.,* 2008).

The cornstarch is a commercial, finely pulverized, the product used for human consumption (Maizena®, Unilever). Its biochemical components (94% carbohydrates) were described in Mazón-Suástegui *et al.* (2008). Maizena® was supplied as a cooked suspension supplement to enrich the natural diet, at a rate of 0.15% of total live weight/day of adults and juveniles in the laboratory tests (Mazón-Suástegui *et al.,* 2008). Briefly, after estimating the amount of cornstarch to be provided, it was dissolved in 0.5 L cold tap water and added to 5 L of boiling tap water. After boiling for 5 min, the suspension was poured into a fiberglass tank with a conic bottom containing 500 L seawater under strong aeration. The dilute suspension was then delivered by gravity flow to the experimental units.

Conditioning of broodstock

One hundred and five adult individuals of *A. ventricosus* with mean 60-70 mm anteroposterior length were collected from at Bahía Magdalena, Baja California Sur, Mexico, and were transported to the laboratory in insulated containers with seawater at 19 ± 0.5°C. An initial histological analysis (beginning) showed that 93% of a sample of 15 specimens were in maturity, spawning, and post-spawning stages.

Table 1. The proximal biochemical composition of tested foods (% dry weight).

	Carbohydrates (%)	Proteins (%)	Lipids (%)	Reference
Tisochrysis lutea	12.3	32.1	22.6	Mazón-Suástegui *et al.* (2008)
Chaetoceros calcitrans	6.9	25.6	34.3	Mazón-Suástegui *et al.* (2008)
Chaetoceros gracilis	19	23.9	8.7	Mallo & Fenucci (2004)
Maizena®	94	0	0	Commercial product label of cornstarch

The scallop's broodstock was conditioned for four weeks in tanks containing 150 L circulating and 5 µm-filtered seawater. The temperature was maintained at 22 ± 0.5°C and salinity of 37.5 ± 0.5. Two treatments with three replicates, each containing 15 scallops, were tested. Treatment one was fed the diet of 4.5×10^9 cells per scallop (*T. lutea* and *C. calcitrans* 1:1) added to the culture water each day. Treatment two was fed the same diet and a supplement of cornstarch, as described earlier.

To measure the reproductive condition at the beginning and end of the conditioning period, a sample of 15 gonads by treatment were analyzed and 1110 histological sections (3 µm) were microscopically evaluated, using hematoxylin-eosin staining. On the basis of the studies of Villalejo-Fuerte & Ochoa-Báez (1993) and Luna-González *et al.* (2000), the reproductive stages of spermatogenesis and oogenesis were classified into six categories (I: undifferentiated or rest, II: initial gametogenesis, III: advanced gametogenesis, IV: maturity, V: mature-spawning (partial spawning), and VI: spawned). The theoretical diameter of oocytes was determined to classify stages of sexual maturity of the ovary, avoiding bias associated with irregular shapes of gametes during oogenesis (Villalejo-Fuerte, 1992; Saout *et al.*, 1999; Román *et al*., 2001).

Nursery management of early juveniles

Young juveniles were obtained at the hatchery by cultivating larvae and postlarvae, using plastic mesh as a settling substrate. Two treatments, each one with three replicates (80 mL of spat) were tested. A first treatment consisted of a microalgal diet (*T. lutea, C. gracilis*, and *C. calcitrans* at a ratio 1:1:1) and a second one was the same microalgal diet supplemented with cornstarch, as described earlier. Spat were grown for 28 days in upwelling cylinders (upwellers) made of fiberglass with a diameter of 30 cm and a false bottom of 800 µm mesh Nytex®. During the experiment, the spats were kept under continuous up-flow of 5 µm-filtered seawater with a daily exchange rate of 300%, a temperature of 22.4 ± 0.5°C, and salinity of 38.5 ± 0.6. Natural and artificial food was available on a continuous basis.

As is done in the nursery units of commercial laboratories, the volumetric method was used to evaluate growth in function of the weekly increase in biomass. Initial volume biomass spat of 1-2 mm (80 mL per replicate) and their weekly increase in volume was determined in each replicate upwellers. This is a usual management during the hatchery production of scallop spat (Mazón-Suástegui *et al.*, 2011). To avoid errors, spat was compacted by swirling the biomass in a graduated cylinder until a constant volume was reached.

Statistical analysis

To evaluate the effect of experimental treatments (diets) on the gonadal conditioning of the scallop broodstock, the difference in frequency (%) of the stages of sexual maturity at the beginning of the process (day 0) and the end of the experiment (day 28), applying a one-way ANOVA. Additionally, the percentage of mature oocytes in the scallops (>39 µm in diameter; Mazón-Suástegui, 2005) was analyzed with one-way ANOVA. In all cases, a Duncan *a posteriori* comparison was developed. To evaluate the effect of experimental treatments on the growth (volume biomass increase) of the scallop juveniles (spat), a Student *t*-test was used to compare results between diets. All tests were performed with a probability set at $P = 0.05$.

RESULTS

Gonadic conditioning of broodstock

We identified five of the six reproductive stages from histological sections, 420 reads at the beginning and 1110 reads at the end of the experiment. The frequency (%) of the stages was different at the beginning and at the end of the treatments (Fig. 1). At the beginning, there were more scallops at stages IV and VI, with a high count of atretic oocytes. After the conditioning period, the scallops in the microalgae + cornstarch treatment had a higher frequency of mature-spawning

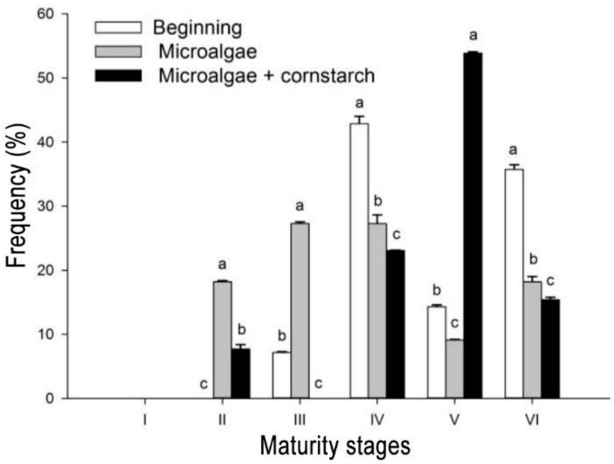

Figure 1. Frequency (%) of maturity stages of *Argopecten ventricosus* at the beginning and after a four-week period of treatments with a microalgal diet (*Tisochrysis lutea* and *Chaetoceros calcitrans*, 1:1) and the same diet supplemented with cornstarch. Maturity stages: I: undifferentiated or rest, II: initial gametogenesis, III: advanced gametogenesis, IV: maturity, V: mature-spawning (partial spawning), and VI: spawned. (Different letters denotes significant differences between treatments).

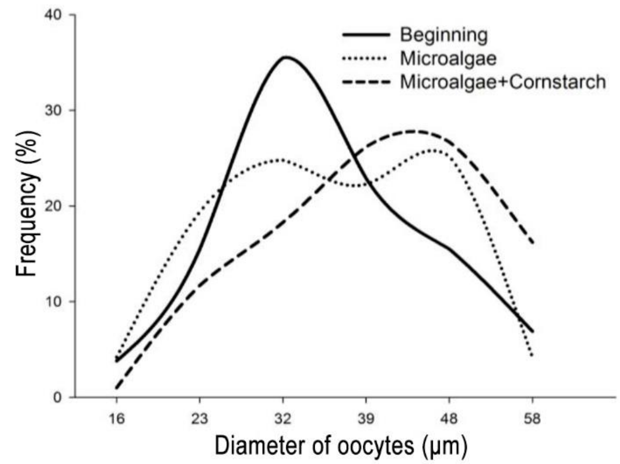

Figure 2. Frequency (%) of oocyte diameter in histologic sections of *Argopecten ventricosus* broodstock at the beginning and after of four-week period of gonadic conditioning in the laboratory, with a 100% microalgal diet (*Tisochrysis lutea* and *Chaetoceros calcitrans*, 1:1) and the same diet supplemented with cornstarch (Maizena®).

stages-V (adding 56.8%), showing a marked significant difference between treatments.

The frequency of oocyte diameter, at the beginning of the conditioning period, was unimodal, in the range 23-36 µm (Fig. 2). After the dietary treatments, the modal frequency distribution is displaced to larger oocytes, generally more than 39 µm, which is the mean diameter of mature oocytes in *A. ventricosus* (Mazón-Suástegui, 2005). The mature oocytes (>39 µm) showed significantly higher percentage in the treatment with a supplement of cornstarch (46.2%, CI95% = 11.4). Although individuals with microalgae alone had higher mature oocytes percentage (32.12%, CI95% = 9.26) than individuals at the beginning of the experiment (22.4%, CI95% = 7.04) the differences were not significant.

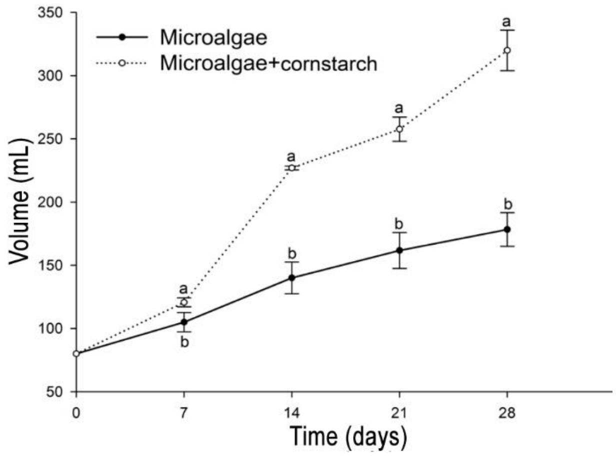

Figure 3. Growth as biomass increase in volume, of juveniles of *Argopecten ventricosus* grown elsewhere on a microalgal diet of *Tisochrysis lutea*, *Chaetoceros gracilis*, and *C. calcitrans* (1:1:1) and the same natural diet supplemented with cornstarch (Maizena®). Different letters denote significant difference between treatments.

Nursery management of young juveniles

The spat fed either diet grew in a linear manner throughout the experiment (Fig. 3), with a steeper slope when the microalgal diet (*T. lutea*, *C. gracilis,* and *C. calcitrans*) was supplemented with cornstarch. At the end of the experiment, the diet containing cornstarch increased 4-fold over the initial volume, reaching 320.0 ± 27.83 mL. This was 80% more than the spat only fed microalgae, reaching 178.3 ± 23.09 mL. The mean absolute increase was significantly different from the first week of the experiment when the spat fed the diet supplemented with cornstarch reached 120.7 ± 6.11 mL and spat fed only microalgal diet reached 105 ± 13.22 mL.

DISCUSSION

Argopecten ventricosus is a microfilter-feeder and demands a food of small caliber, which captures by filtration of important volumes of water. Its gill filaments allow it to retain planktonic organisms of animal or plant origin, bacteria, particulate organic detritus, and even dissolved organic substances normally present in seawater (Mazón-Suástegui, 1988). Despite their broad trophic spectrum, bivalves are specialized herbivores and phytoplankton is their typical diet. They digest it through the digestive gland, which produces proteases and the crystalline stylet, an enzymatic structure that releases amylases and celluloses by rubbing against one or more chitinous shields present in the diverticulum (Pereira, 1993).

The microalgae mostly used in the cultivation of bivalve mollusks are provided in mixtures of two or three species to improve the nutritional profile of each stage of cultivation in the laboratory. Adding cornstarch to the commonly used microalgal diet can substantially improve the maturation process of adult scallops during gonadic conditioning in the laboratory (Mazón-Suástegui, 1988). It also enhances the growth of early juveniles of several bivalve species during the nursery period in the laboratory (Mazón-Suástegui *et al*., 1988).

By definition, the gonadic conditioning technique allows to accelerate, maintain or delay maturation in bivalve mollusks, by controlling the most relevant parameters of the gametogenic cycle, mainly temperature and feeding, to obtain spawners at different times of the year (Mazón-Suástegui, 2005). With the microalgal diet enriched with Maizena®, a breakthrough in the process of sexual re-maturation of the brooders was attained within a short period of four weeks. Considering that the experiment was started with the scallops in stages of maturity, spawning and post-spawning, it is assumed that the mixed diet favors the sexual maturation and rematuration. This confirms that a high carbohydrate content in the microalgal diet enriched with cornstarch favors the synthesis and accumulation of glycogen in the soft parts of mollusks (Willis *et al*., 1976), which can be subsequently used for the formation of sexual products (Turgeon & Haven, 1978).

At the end of the experiment, we were able to verify the above-mentioned statements. The scallops receiving a supplementary diet with cornstarch increased the proportion of mature-spawning stages by almost 4-fold (14.3% at the beginning and 53.8% at the end of the trial). The scallops also showed a significantly higher proportion of mature oocytes (>39 µm in diameter) per individual. This is highly effective for spat production since this is the most appropriate phase for spawning induction and there are a greater proportion of viable oocytes for release and fertilization. Results suggest that cornstarch in the diet during maturation of broodstock will lead to more competent eggs and a larger number of individuals ready to spawn. This could increase the genetic diversity in offspring and minimize inbreeding scenarios.

The scallop *A. ventricosus* reproduces year-round if there is enough food available (Luna-González *et al.*, 2000). This strategy for reproduction, along with a quick recovery of reproductive maturity (30-40 days), is ideal for continuous larval production. Assuming that gonadic conditioning of adults under controlled conditions generates higher percentages of adults suitable for spat production (Mazón-Suástegui, 2005).

In addition, the use of cornstarch as a supplement is an improvement for reproductive fitness, as the juveniles that received cornstarch improved growth substantially (80% more than the spat only fed with microalgae alone), shortening cultivation time to reach a size suitable for grow-out in open water.

Although the contribution of cornstarch comes from the addition of carbohydrates, the results indicate that the reproductive condition in adults and growth of spat was significantly improved, suggesting that more carbohydrates in the diet play an essential metabolic role. This argument is supported by Racotta *et al.* (1998) and Ruiz-Verdugo *et al.* (2000) who found an increase in carbohydrate content during the breeding cycle. It also follows that cornstarch in the diet provides energy for reproduction, since carbohydrates can be used as precursors of lipids in vitellogenesis (Gabbot & Bayne, 1973; Zandee *et al.*, 1980; Barber & Blake, 1985; Pronker *et al.*, 2008) and energy for sperm motility (Arellano-Martínez *et al.*, 2004).

These results support similar findings of other mollusks of commercial interest, including *Modiolus capax*, *Pinctada mazatlanica*, *Pteria sterna*, *Pinna rugosa*, *Crassostrea corteziensis* (Mazón-Suástegui, 1988; Mazón-Suástegui & Aviles-Quevedo, 1988; Perez-Camacho *et al.*, 1998; Mazón-Suástegui *et al.*, 2008, 2009; Mamat & Alfaro, 2014).

Studies on optimization of the dose and rate of cornstarch to the normal microalgal diet should establish a cost-effective feeding protocol during gonadic conditioning of adults and nursery management of young juveniles of several species of bivalves.

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