Developing Hatchery Methods for the Mangrove Oyster, *Crassostrea corteziensis* for the Pacific Coast of Mexico

Indigenous Species Development/Study/09IND01UH

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ABSTRACT

Crassotrea corteziensis is an important oyster culture species in the Mexican State of Nayarit where it forms the basis of a thirty-year old industry. This industry is based on spat collection, which has been primarily successful only in the State of Nayarit. This limits its potential for other areas of Pacific Mexico. This investigation was designed to develop the capacity and methods to reliably produced eye-larvae in a hatchery based at the School of Marine Sciences (FACIMAR) at the Autonomous University of Sinaloa, Mazatlan Campus. This hatchery and associated training and demonstration farm would allow FACIMAR to conduct research and provide training to a wide range of stakeholders.

Most of the objectives of the investigation were achieved, including development of a successful microalgae production facility and successful broodstock conditioning system. High mortality of the larvae occurred due to bacterial and protozoan infections, which prevented production of spat for the community groups involved in this work. The bacterial infections are believed to be associated with water pollution in the waters surrounding Mazatlan. Work will continue to improve the water treatment system at FACIMAR and larviculture will be attempted again in early 2012.

INTRODUCTION

Mexican aquaculture is focused on shrimp culture, although diversification of aquaculture has been identified as a national development priority. Bivalves offer a multitude of possibilities to diversify aquaculture if specific obstacles can be addressed. Oyster production is currently stable, producing

between 40,000 to 50,000 tons annually with the Gulf of Mexico industry based on *Crassostrea virginica* representing 90% of this (CONAPESCA 2009).
Oyster production on the Pacific Coast of Mexico depends on two introduced species, the Pacific Oyster (*Crassostrea gigas*) and more recently, the Kumamoto Oyster (*C. sikamea*). Both species readily adapt to local conditions and grow rapidly. Much of the seed for these species is imported from the United States, and local seed production has been decreasing due to disease and temperature issues (CIAD 2003; Tapia et al. 2008). *C. gigas* also suffers from production issues related to its tendency to become "spawny" or "thin" during the reproductive and post-reproductive season. Mortalities also occur during the warmer months and may be related to the "summer mortality" phenomena observed for this species in the Pacific Northwest of the United States.

C. corteziensis, on the other hand, does not suffer from these issues and has a high level of local acceptance. It has been cultured primarily in Nayarit State for over 30 years but expansion of the industry locally, and to other areas in Mexico, is hampered by lack of hatchery production. This species has been produced on a limited scale in a few hatcheries previously, but techniques were considered proprietary information.

The objectives of this investigation were therefore to develop methods to condition *C. corteziensis* broodstock and for hatchery production of eye-larvae. This also included establishing a microalgae production facility at FACIMAR/UAS and training staff and students in all methods. It was also hoped that a small demonstration farm could be established in the waters adjacent to the FACIMAR facilities for research and training. If successful, this would enable FACIMAR staff to produce larvae for research purposes, and to supply small community-based farms. Training courses and student classes would utilize these facilities. Methods would also be shared with several stakeholders, including a cooperative shrimp hatchery, to encourage private sector development of oyster hatcheries.

METHODS

Development of the hatchery

The School of Marine Sciences (FACIMAR) at UAS is an education institution conducting research, extension and academic training in marine sciences and aquaculture. It is located in Mazatlan, Sinaloa, Mexico. The facility is adjacent to the main seawall of the city (Figure 1). Three small laboratory facilities were developed at FACIMAR: 1) hatchery; 2) microalgae production facility; and 3) a broodstock conditioning system.

Study 1: Evaluation of the adequacy of adjacent water areas for holding oysters.

Juvenile *C. corteziensis* were obtained from farms and distributed in mesh bags, which were hung on a long line in the area adjacent to FACIMAR to determine if these conditions were adequate for conditioning and maintaining broodstock (Figure 2). Growth was monitored for 5 months. Water quality parameters such as temperature, salinity, pH and turbidity were also monitored for the same period of time. Bacteriological analysis was also conducted for *Vibrio* and coliform bacteria in oyster tissues and water.

Collection and maintenance of oyster broodstock

Approximately 100 adult oysters were collected from different locations and kept in the oyster conditioning system at FACIMAR. This consisted of recirculation tanks with salinity at 30 ‰ and temperature at 24 ºC (same as the collection sites). Aeration was also provided. Feed consisted of three species of microalgae, *Isochrysis sp., Tetraselmis* sp. and *Chaetoceros sp*. These were supplied in three daily rations of 200,000 cells per ml, supplemented with a 0.5 g daily ration of mixed corn starch and rice flour. The tanks were cleaned before the first feeding of the day and the entire system was cleaned weekly. Figure 3 shows aspects of the broodstock and microalgae systems.

Figure 1. Location of the School of Marine Sciences (FACIMAR) in Mazatlan, Sinaloa, Mexico. Arrows indicate the location of the hatchery and oyster holding areas.

Figure 2. Transplanting of oyster broodstock to oyster holding area near FACIMAR (a and b); students and staff measuring oysters (c and d).

Figure 3. Collecting broodstock (a), recirculation system for holding broodstock (b), microalgae culture in 20 liter containers (c) and larger batch culture (d).

Microalgae culture

Microalgae cultures were started in test tubes and the volume was scaled up to 20 liters, using standard algae culture methods. The 20 liter cultures were used to inoculate 900 liter semi-batch culture vessels (Hoff and Snell 1987) (Figure 3).

Spawning

Two methods were used for spawning: thermostimulation and strip spawning. The first utilized rapid temperature changes varying between 18 and 20 ºC, with changes every 30 minutes. When oysters began to spawn, they were separated into separate containers with clean, filtered seawater. When spawning was completed, sperm was added to the eggs for fertilization. When broodstock failed to respond to thermostimulation, these were sacrificed, opened and the gonadal tissue excised using gentle scraping with a scalpel.

Larviculture

D-stage larvae were transferred into 200 liter tanks filled with filtered seawater at an initial density of 8 larvae per ml. Feed consisted of *Chaetoceras* sp. (2 million cells/ml/day). Light aeration was also supplied. Larvae were sieved out every forty-eight hours, the tanks cleaned and the water was exchanged (Figure 4).

Figure 4. Induction of spawning (e and f); sieving larvae (g and h) and microscopic vie of D-stage larvae (i).

RESULTS

Evaluation of oyster holding area in adjacent waters

The oyster kept in waters adjacent to FACIMAR showed no significant growth either in length, width or weight (Figures 5, 6 and 7). Weight was maintained at 12.5 g and length at 4.4 cm over the five month period. Survival was low at 82% of the initial count after 5 months of holding.

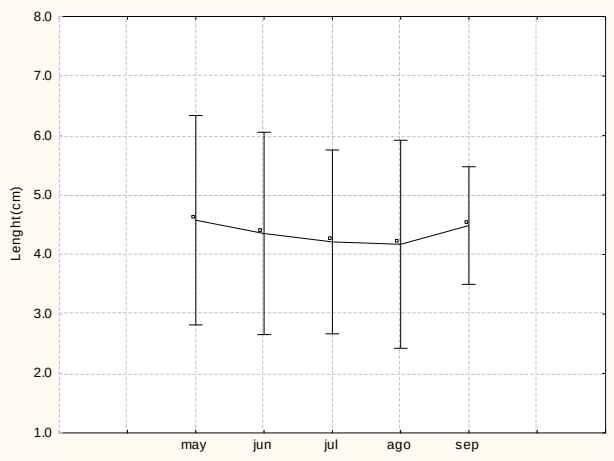

Figure 5. Monthly mean lengths (DVM) for oysters held near FACIMAR.

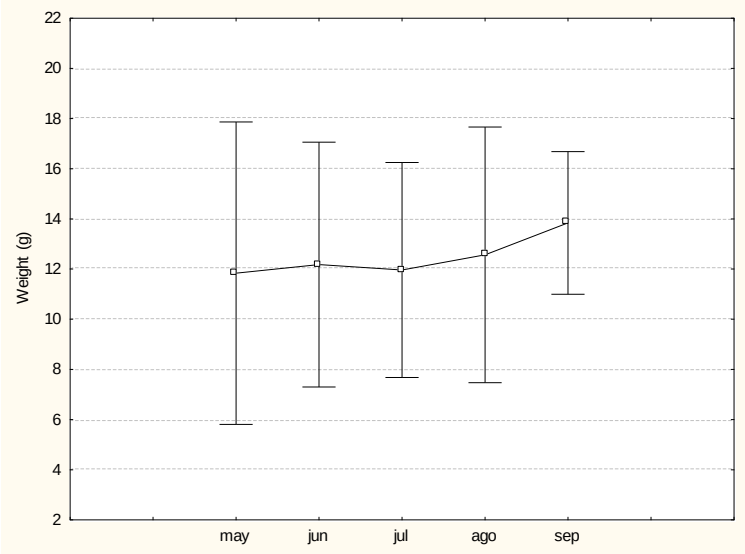

Figure 6. Monthly mean weights for oysters held near FACIMAR.

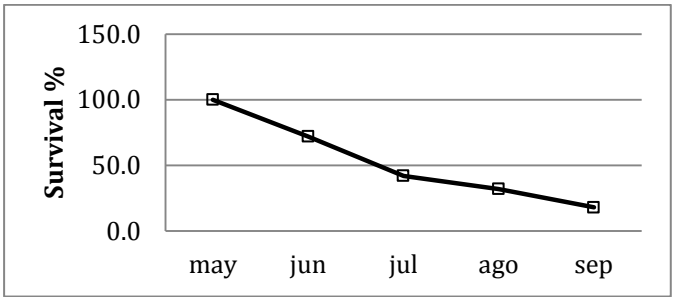

Figure 7. Monthly survival of oysters held near FACIMAR.

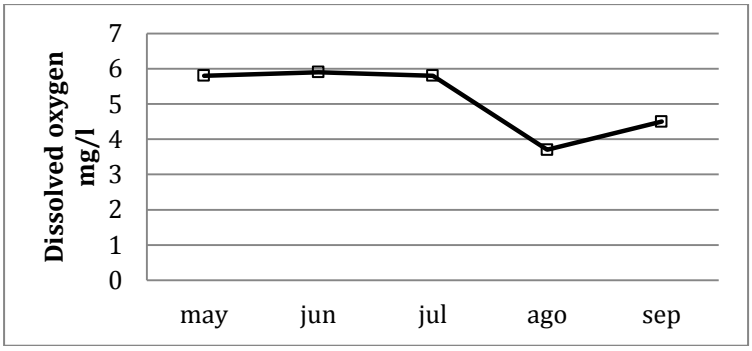

Figure 8. Dissolved oxygen levels at FACIMAR oyster holding area.

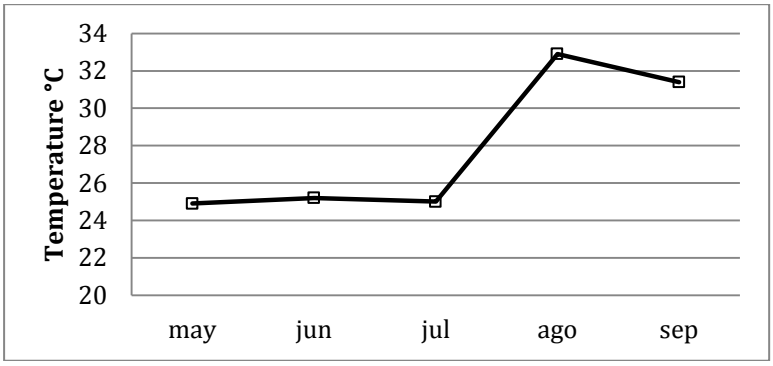

Figure 9. Mean monthly sea surface temperature at FACIMAR oyster holding area.

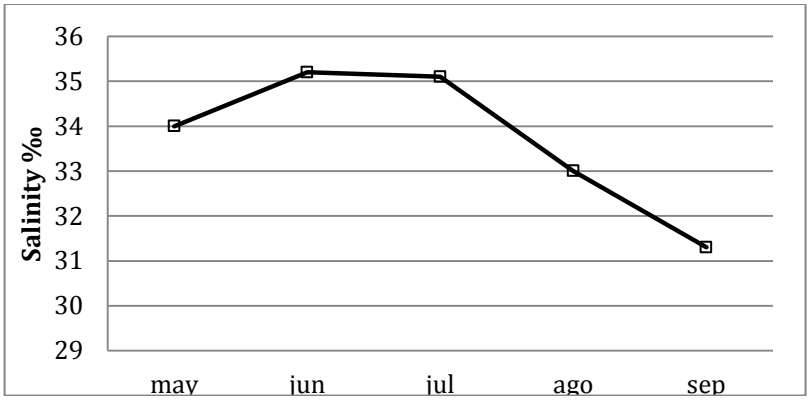

Figure 10. Mean monthly salinities for FACIMAR oyster holding area.

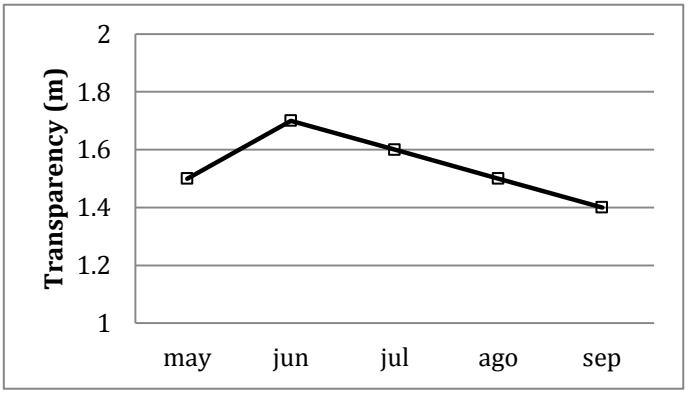

Figure 11. Mean monthly turbidity at FACIMAR oyster holding area.

Dissolved oxygen levels were lowest in August (3.7 mg/l) and September (4.5 mg/l) (Figure 8). From May to July, water temperatures were stable at 25 °C, and then rose in August and September. The maximum temperature occurred in August (32.9 °C) (Figure 9). Salinity rose from May to June (35.3‰) then declined to 31.3 ‰ in September (Figure 10). Turbidity had a mean value of 1.5 m during the five month period (Figure 11).

Vibrios and coliform bacteria in oyster tissues and seawater

Table 1 shows Vibrio values for oyster tissues and the seawater at the FACIMAR holding area. Yellow colonies were particularly prevalent.

Table 1. Results of Vibrio analysis

		UFC/ g in oyster tissues		UFC/ml in seawater	
Month	**Sample no.**	**Yellow**	**Green**	**Yellow**	**Green**
MAY	1	60333	265833	167	0
	2	333	0	0	0
JUN	1	0	333	0	0
	2	5000	2333	0	0
	3	0	0	0	0
JUL	1	333	167	25000	0
	2	2167	0	0	0
	3	0	0	0	0
AGU	1	167	0	0	0
	2	0	0	167	0
	3	0	0	1167	0
SEP	1	0	0	0	0
	2	52500	0	X	X

Table 2 shows results from testing for coliform bacteria (total and fecal) in oyster tissues and seawater. Most of the higher counts exceed the regulatory standards for acceptable limits (NOM031-SSA1-199) in shellfish growing areas.

Broodstock conditioning and maintenance

Broodstock conditioning in the recirculation system was successful before and after spawning. The mixed microalgae diet of 200,000 cells/ml along with a 0.5 g supplement of corn starch and rice flour was adequate to achieve maturation of gametes. The temperature of 23 °C and 25 ‰ salinity also appeared to be adequate.

Larviculture

Although larvae were consistently produced using thermostimulation to induce spawning, several attempts to raise larvae to the setting stage all failed with mass mortalities of larvae observed concurrently with the appearance of protozoans and other ciliated organisms. Larvae also showed signs of bacterial infections.

Table 2. Total and fecal coliform bacteria in seawater and oyster tissues.

		Seawater		Oyster tissues	
Month	Sample no.	NMP/100ml Total	NMP/100 ml Fecal	NMP/100 g Total	NMP/100 g Fecal
MAY	1	>2400	15	>2400	0
	2	215	20	507	98
JUN	1	>2400	2050	>2400	>2400
	2	>2400	45	>2400	0
	3	850	>2400	>2400	>2400
JUL	1	70	70	721	298
	2	1750	1750	426	315
	3	>2400	70	>2400	375
AGU	1	1100	>2400	482	129
	2	1750	35	>2400	>2400
	3	110	25	>2400	192
SEP	1	>2400	>2400	1345	350
	2	X	X	>2400	>2400

DISCUSSION

The high mortality rate and lack of growth exhibited by the oysters kept at the FACIMAR oyster holding area demonstrate that this site has inadequate conditions to maintain oysters. Hence, establishment of a training and research farm at this site is not possible. Given that the temperatures were within the range considered conducive to the species (21 to 31 °C) and that salinities (31 to 39‰) were also within the documented tolerances (Gongora Gomez et al. 2006), lack of adequate food may be to blame. *C. corteziensis* is an estuarine species and needs a high level of phytoplankton to thrive (Chavez Villalba et al. 2008). The mean secchi disk reading of 1.5 m suggests that this water has low levels of primary productivity (Fench et al. 1982; Shapiro et al. 1975).

An additional indication of the poor water quality of the holding area is the high levels of *Vibrios* and coliform bacteria. While species level identification was not possible, certain colony colors often indicate well known groups. In particular, the predominance of yellow colonies of *Vibrio*s are indicative of species which can harm oysters or affect humans (e.g. *V. alginolyticus*, *V. tubiashii*, *V. fluvialis*). Green colonies may include *V. parahaemolyticus, V. vulnificus, V. damsel* and *V. mimicus.* The first two can be injurious to humans. Vibriosis in general is well known to be a cause of mass mortalities in bivalve larvae hatcheries (Caceres-Martinez and Vazquez-Yeomans 2001).

The failure to produce larvae to setting stage is discouraging, but further attempts to improve water treatment to eliminate bacterial sources of contamination in the source water, as well as to eliminate contamination of the algae cultures will continue in early 2012. The basic facilities and capacity to operate a small research hatchery were successfully established during this work. Efforts will also be made to locate another location for the research and demonstration farm. Four CRSP-sponsored students were also trained as part of this research.

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