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RESEARCH ARTICLE

Variations in the reproductive cycle of the oyster *Crassostrea virginica* (Gmelin, 1791), Pueblo Viejo lagoon, Veracruz, Mexico

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Abstract

1 - Mexican oyster production in the Gulf of Mexico has fallen drastically in most coastal lagoons, whereas Pueblo Viejo Lagoon production (state of Veracruz) has been sustained, providing a source of seed for the restocking of other lagoons. This has been achieved due to bed management carried out by local fishermen. This management is based on empirical knowledge of seed collection from planted shell beds.

2 - This study describes variations in gametogenic activity and the reproductive cycle during the year 2002, with a view to improving shell laying programs. Samples were taken from two beds, subject to different environmental and pollution conditions. Malagana point, with a heavy organic load and a strong tidal current, and Matacuaya Island, with a low organic load and no current.

3 - Thirty oysters over 8 cm shell length were sampled monthly from each bed for histological analysis of the gonad tissue.

4 - There were variations in gonad activity between localities, which are reflected in the gametogenic cycle and spawning. At both stations spawning extends throughout the year, but in larger numbers from June to October at Malagana, contrasting with a low frequency for most of the year and one intense spawn during July and August at Matacuaya.

5 - These differences in the gametogenic cycles and spawning periods are a result of population recovery capacities under different environmental conditions. The Malagana population is exposed to a higher organic load from the Panuco River in addition to urban discharge, resulting in shorter post-spawn and rest periods, faster gametogenesis, and a well defined mature stage with a high intensity spawning period and three peaks. Meanwhile, the oyster population from Matacuaya presented longer, more intense post-spawn and rest stages; gametogenesis extended longer, in a lower percentage of the population, with a brief maturity period.

6 - This differential reproductive behaviour has positive implications in recruitment, giving the oyster populations from Pueblo Viejo lagoon the opportunity to take advantage of every settlement possibility throughout the year. However, the higher organic load that enhances reproduction at Malagana poses a health risk, given the high fecal bacteria count and heavy metal concentration in the sediment, constantly re-suspended by the tidal current flux.

Keywords: Reproduction, American oyster, *Crassostrea*, pollution, Population biology

Introduction

Mexican oyster production reached its peak at 59 600 tons in 1989, declining to 25 800 tons in 1993. It has gradually recovered due to management practices in the states of Tabasco and Veracruz. Historically the Gulf Coast of Mexico has produced from 88 to 92% of Mexican oyster production. Veracruz state led production until 1996, when Tabasco overtook it, followed by the states of Tamaulipas and Campeche. These volumes are not a result of production potential based on total lagoon surface; Tamaulipas has 274 736 hectares, followed by Campeche with 198 500 hectares, Veracruz with 193 300 hectares and Tabasco with 27 400 hectares. The dominant position of Tabasco State, with 70% of production in just 4% of lagoon surface, is an example of oyster production potential in the Gulf of Mexico's Mexican coast, which in theory could be increased to over 500 thousand tons (SAGARPA, 2004). The decline and low production rates in some states have been attributed to several factors: a) overfishing and mismanagement of beds; b) environmental degradation of coastal lagoons; c) pollution. Environmental factors and pollution have an impact on oyster bed production, reflected in growth, mortality and reproduction, enhancing or decreasing gonad development and the reproductive cycle, acting directly on the duration and intensity of gametogenesis and spawn (Galtsoff, 1964, Loosanoff, 1968, Baqueiro, 1998), or indirectly by weakening oyster resistance to parasites and disease (Lauckner, 1983, Perkins, 1993). Coastal lagoons on the Gulf of Mexico have been severely affected by different human activities such as intense fishing, water flow modification, and several types of pollution (Contreras, 1985). However, the causal link between the collapse of fisheries and human activities has not yet been clarified. Annual oyster production in Pueblo Viejo Lagoon has remained stable at around 4 800 tons. It has been used as a source of oyster seed for restocking other lagoons, even though levels of pollution detected are among the highest in all the coastal lagoons (Villanueva & Botello, 1992, 1998)

As one of the most important fishery resources of the Gulf coast, various management and aquaculture practices have been implemented in the past (Ramirez & Sevilla, 1965; Aldana Aranda, 1988). Reproductive studies are scarce, considering the high variability of this characteristic that depends on environmental conditions (Davis & Chanley, 1955). Sevilla & Mondragón (1965) were the first to study reproductive cycle in Mexican waters in oyster populations from the Tamiahua lagoon, Veracruz, reporting a seasonal reproductive cycle with well defined rest, gametogenic, spawning and post-spawn periods, without overlapping, coinciding with Ortega and Arroyo (1988) who observed that spawning is limited to July and August at the same lagoon. Rogers and García-Cubas (1981), and Martínez et al. (1995) described the reproductive cycle in Terminos Lagoon, Campeche, reporting two spawning periods during spring and fall. George et al. (2003) reported mature organisms in the Mecoacan lagoon, Tabasco from June to January, with a peak during December, while spawning extended throughout the year, with the exception of July and August. Given the diverse environmental conditions and the impact of urban pollution in some localities, the objective of this study was to identify variations in the reproductive cycle and gametogenic activity in the Pueblo Viejo lagoon, where some oyster beds are subject to intense pollution and others are relatively clean.

Methods

Sampling sites

Pueblo Viejo Lagoon is located on the west side of the Gulf of Mexico in the state of Veracruz; it is part of the alluvial plain of the Panuco River, latitude 22° 11' N and 22° 12' N and longitude 97° 50' W and 97° 57' W, with a total surface area of 93.7 km^2. The climate is sub-tropical, with an average rainfall of 1507 mm distributed 90% in summer and 10 % during winter. Sediment composition consists of sand and silt with shells (Contreras, 1985). Five small rivers flow into the lagoon with significant runoff only during the rainy season; the lagoon is permanently connected to the Panuco River by the Malagana canal that opens one kilometre from the river mouth to the Gulf of Mexico. The town of Pueblo Viejo is located along this canal, while the ports and city of Tampico are located on the banks of the neighboring Panuco river (Figure, 1). Two sampling sites were selected for this study, one on the mouth of the Malagana canal, constantly influenced by the Panuco River and domestic waste discharge from Pueblo Viejo, and the other in the western-central part of the Matacuaya lagoon, where water conditions represent the average of the main body of water (Fig. 1). Oyster beds are managed and commercially exploited to some extent at both localities, supporting the two main oyster beds of the lagoon. No environmental data were recorded during sampling; therefore data relating to the Malagana station were obtained from the Oceanography station (OS), corresponding to the period 1996 and 1997 and for the Matacuaya station, data from the National Water Commission (Comision Nacional del Agua, CNA) taken during 2002 were used (Table 1). Table 2 presents sediment quality data for Malagana, over the period 1996-1997 (OS, personal communication).

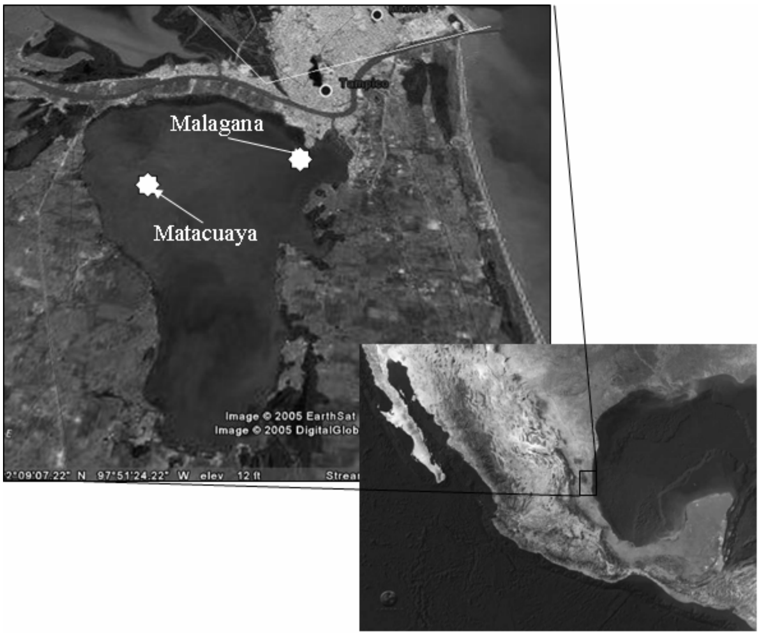

Figure 1. Sampling sites at Pueblo Viejo Lagoon, Veracruz, Mexico.

Table 1. Water quality data for Punta Malagana oyster bed (1996-1997) (Oceanografía, Pers. Comm.) and Malagana oyster bed (2002) (Comision Nacional del Agua, Per. Comm.), Pueblo Viejo Lagoon, Veracruz, Mexico.

		Spring		Summer		Fall		Winter	
		Malagana	Matacuaya	Malagana	Matacuaya	Malagana	Matacuaya	Malagana	Matacuaya
NH_4	mg-at.l^{-1}	-	0.22	0.38	0.02	4.03	0.01	0.24	0.02
NO_3	mg-at^{-1}	12.67	0.002	8.83	0.01	3.28	0.002	4.7	0.006
NO_2	mg-at^{-1}	-	0.003	0.3	0.025	0.02	0.2	0.17	0.006
PO_4	mg-at^{-1}	0.81	0.09	0.07	0.11	0.02	0.06	1.15	0.03
Fecal Col.	cel/100ml	500	0	2200	0	2000	32	6000	111
COD	mg^{-1}	759.2	1.6	158.69	0.1	4.7	2.9	1000	2.8
Chlorophyll		-	6.5	-	4.7	-	9.4		3.4

Sampling methods

Sampling was conducted from January to December 2002, on a monthly basis, excluding November. Thirty oysters with a shell height over 8 cm, were collected monthly at each location. Oysters were cleaned, separated at the site and transported to the laboratory for further analysis. The soft parts of the oyster were individually wrapped in gauze and labelled for identification. Samples were fixed in Bouin for 24 hours, after which they were rinsed in running tap water for 12 hours. A 1 cm^3 section from each oyster was dehydrated in Hystocassets™ with alcohol, cleared with Clearene™ and embedded in Paraplastic™ 53° - 56°C melting point. Microtome sections 7 µm thick were stained with Haematoxylin – Eosin (Luna, 1968).

Gonadic stages were classed according to Lucas (1965): Stage I: Rest - gonad tissue is hardly evident, represented by a thin layer of cells lying parallel to the outer wall among reticular connective tissue, gametogenic activity may be present, or germinal cells from previous seasons may persist, but in any case cases it is not possible to identify the sex. Stage II: Gametogenesis - gametogenic activity is evident, with follicle enlargement, germinal cells are differentiated. Depending upon the advance of gametogenesis, mature gametes may or may not be present, sexes are clearly differentiated. Stage III: Mature - dominance of mature gametes, ova are densely packed presenting a polyhedral shape; sperm are densely packed, forming bundles with flagella towards the centre of the follicle. Gametogenesis frequently persists through maturation and spawning. For this study, only organisms with over 80 % of follicles full of mature gametes were considered to be mature; Stage IV: Spawn - follicles are partially emptied, ova lose their polyhedral shape, sperm are found in the centre of the follicle, forming an irregular mass; peripheral follicles lose their germinal tissue and become ciliated ducts where only mature ova or sperm may be observed (Galtsoff, 1964), gametogenesis may continue through this stage; Stage V: Post-spawn - follicles are anastomosed, broken and empty, with the exception of a few, left over, mature and immature cells, abundant phagocytes are present in and among follicles. In order to determine the prevalent reproductive stage, a minimum of six gonad sections per oyster were observed under the microscope. The prevalent stage was assigned with over 60 % dominance in the gonadic tissue (follicle area and number of follicles). A variance analysis (F statistical test) was carried out between months and reproductive stages in order to ascertain the significance of differences. For the monthly maturity analysis, a value of 0 to 90 was given to the reproductive cycle, with a mean value of: Rest 10; Gametogenesis 30; Maturity 50, Spawn 70 and Post-spawn 90, which allowed results to be handled as a frequency distribution. For the annual cycle, the original number of organisms was used.

Results

Water quality at Malagana station shows a heavy organic load with high fecal bacterial counts throughout the year, contrasting with Matacuaya station, where a typical lacunar organic and nutrient load was reported, with a low fecal bacterial count only during fall and winter (Table 1). Table 2 presents sediment characteristics at Malagana during 1996 – 1997. Sediments were sand-silt, with a low percentage of organic matter, but high heavy metal content. Sediment re-suspension was evident during the north seasons (fall and winter) at Matacuaya, and throughout the year, on a daily basis with tidal currents at Malagana.

Reproductive cycle at Matacuaya. (Fig. 2)

Organisms at rest were observed throughout the year, with the exception of August. The highest percentages were registered in January, 47%, and December 38%, with an annual mean of 21%. Gametogenesis extended from January until July, with 50% during April and May; a small percentage (5%) was observed during November and December, the result of male activity, which was not registered in females.

Table 2. Sediment quality data for Malagana, Pueblo Viejo Lagoon, Veracruz, Mexico. (1996-1997) (Oceanografía, Per. Comm.).

		June13, 1996 Spring	September 18, 1996 Summer	December 3, 1996 Fall	March12, 1997 Winter
Density	gr/cm3	1.56	1.65	1.50	1.85
pH	UpH	8.30	9.30	8.28	8.25
Carbonates	%	26.75	18.98	28.95	77.11
Organic matter	%	7.73	0.44	0.98	0.51
Cu	mg/kg	22.49	18.74	149.76	4.65
Cr	mg/kg	43.41	26.89	21.92	16.27
Fe	mg/kg	15465.00	36330.00	10453.00	9112.0
Ni	mg/kg	59.19	26.07	58.47	11.62
Pb	mg/kg	41.22	48.34	575.05	190.61
Fecal bacteria.	nmp/100g	9265.	500.00	2700.00	1300.00
BOD	$mg^{-1}l$	134.50	45.30	248.00	79.30
QOD	mg^{-1}	13580.00	18329.00	1123.00	13700.00
Mean grain	phi	5.72	5.72	5.46	1.72
Calsification	phi	1.39	1.89	1.43	1.74
Skewness	phi	0.42	0.42	0.83	0.08
Sand	%	16.13	25.64	6.10	91.17
Silt	%	70.66	61.54	66.47	6.94
Clay	%	13.21	12.82	27.45	1.88

Maturity was registered in a very low percentage of the population, with a mean of only 4% and a maximum of 15% during April and June; in males, it was limited to the months of May, June and August, whereas in females it extended from January to June, in a slightly higher number of organisms (15%). Spawning was registered from February to December, with a maximum during August in 95% of the population and two minor pulses in February and April-May of 30% and 25% respectively, with an annual mean of 32%. Females characterize this stage, with mass spawning from July to September, with a peak of 60% during July and an annual mean of 24%. However, the male population presented three pulses with lower intensity: in February, 5%; April, 10% and August, 40 %. Post-spawned organisms are present throughout the year, with the exception of April and August, with a very close resemblance to spawn, but with peaks a month or two later: January, March and June (29%), September and October (40%) and December (52%), representing a mean of 29% of the population over the year. Females dominate this stage with up to 65% during November, four months after the main spawning peak. Males showed four small pulses of only 15%.

Reproductive cycle at Malagana.- (Fig. 3)
Rest at this locality was seasonal, with a main pulse from October to February, up to 90% from January to December, and an isolated 20% during May. Gametogenesis started in January, growing in intensity until March (60%), dropping to a mean of only 10% until August. Female gametogenic activity was limited to two pulses: February–March (35%) and July–August (7%); whereas for males it was continuous from January to June, with a mean of over 10% and a peak of only 30% during March. Maturity extended from February to September, with a peak of 78% during April of 78%, and a mean of over 15% throughout the year, dominated by females that presented a maximum of 60% during April; there was a slight similarity to males, in that they presented maturity over the same period, but with a maximum of just 17%. Spawning extended from February to November, with three pulses, June (70%), August (79%) and October (67%), and an annual mean of 35%. Females presented the longest and most intense spawn, with three peaks in June, August and October at 50%, 50% and over 60% respectively, with a mean of 25%. While the male population spawned in two pulses: February-March at 10% and May to September, with a peak of 29%. Post-spawn was

registered from May to December, with three peaks: September (52%), July (31%) and November (25%), annual mean of 14%. For females, a minor pulse was recorded during June-July (16%) and a major pulse from September to December, with a peak in September (43%). Post-spawn was limited to two pulses in males, 5% during May and 16% in July, extending until September.

Sex ratio

At both localities there is female dominance over males, 1.8 females per male at Matacuaya and 1.7 at Malagana, with the exception of the months of January and May at Matacuaya, and January, March and May at Malagana, when there is a 1:1 sex ratio.

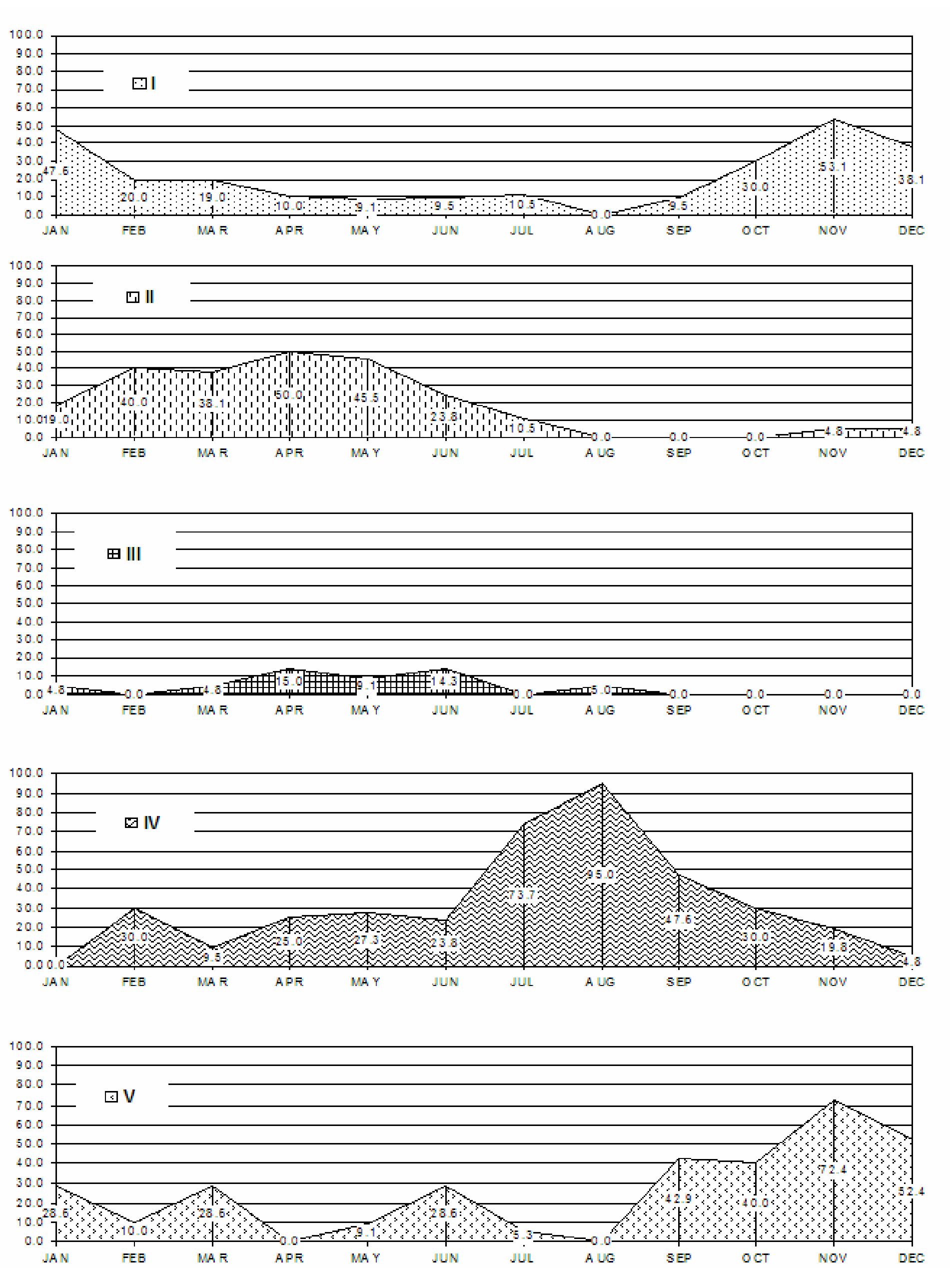

Figure 2. Diagrammatic representation of the reproductive cycle of *Crassostrea virginica* from Matacuaya Island, Pueblo Viejo Lagoon, Veracruz, Mexico. I rest, II gametogenesis, III mature, IV spawn, V Post-spawn.

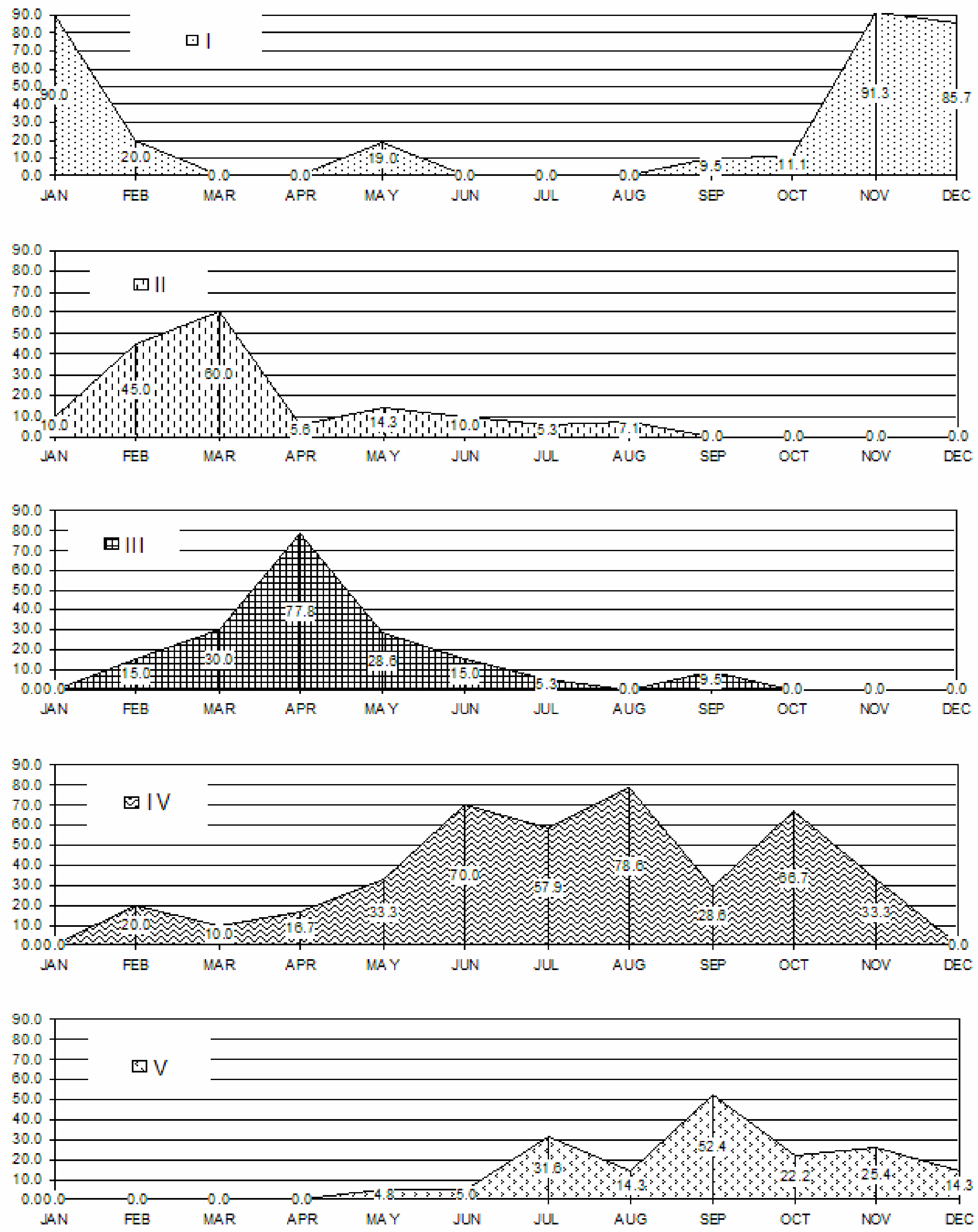

Figure 3. Diagrammatic representation of the reproductive cycle of *Crassostrea virginica* from Malagana point, Pueblo Viejo Lagoon, Veracruz, Mexico. I rest, II gametogenesis, III mature, IV spawn, V Post-spawn.

Discussion

The Panuco River and the urban development of Pueblo Viejo and Tampico contaminate the oyster beds with a heavy organic load, possibly caused by sewage discharge, generating high bacterial counts and heavy metal deposition in sediments that, while enhancing reproduction, also favor the bio-accumulation of pollutants in the oysters. This pollution is evident at Malagana station but not at Matacuaya station.

At both localities, spawning extended from February to November, but with differences among stages. There were differences in the intensity and duration of the stages, even among sexes from the same locality. The differences illustrated in figures 2 and 3 are confirmed in table 3, where rest and maturity showed significant differences below 0.005%, gametogenesis close to 10 %, while no significant difference was detected for spawn, above 0.05% was registered for post-spawn. These differences reflect the number of organisms at each reproductive stage, but do not reflect the duration of the stages; differences in the duration of stages are recorded in Table 4, that shows a significant monthly difference between localities.

Local conditions determine gonad maturation and the spawning process, as well as rate of recovery, which is more demanding in females than it is for males (Galtsoff, 1964). Contreras (1985) reports a maximum depth of 1.5 m in the lagoon; depths registered at sampling sites were 1 m for Matacuaya and 2.5 for Malagana. Although no registers of currents are available, it was observed that tidal currents are strong in the Malagana channel, given its proximity to the mouth of the lagoon. At Matacuaya no current was recorded during sampling periods. Differences in organic load among localities, represented by the COD, and levels of Nitrates and Nitrites (Tables 1), provide evidence of the influence of the Panuco River and the urban area in the quality of water from Malagana.

Higher flow rates and organic load at Malagana enhance faster gonad recovery and maturation, with an extended spawning period, that includes over 60% of the reproductive population over two months, February and March, extending until August. While at Matacuaya, environmental factors induce slower gametogenic activity that extends to a lower percentage of the population over seven months, not allowing a clear maturity stage.

Maturity is a characteristic stage of massive synchronous spawning populations, which becomes less evident as spawning becomes more continuous. Spawning is the population's response to environmental factors; it can range from massive, synchronous and intense, to continuous, low intensity and partial, leaving traces in the post spawn stage, depending on gonad recovery capabilities (Baqueiro, 1998).

Sevilla & Mondragon (1965) and Ortega & Arroyo (1988) reported that reproductive stages for oyster populations from Tamiahua are limited to two or three months, and replace each other (not simultaneous). However, in this study, all stages are present throughout the year, in particular the spawning period, which extends over several months. This coincides with reports by other authors for different coastal lagoons from the states of Yucatan, Campeche, Tabasco and Veracruz, (Rogers & García-Cubas, 1981; Hernádez & Ortega, 1988; Navarrete Rosas, 1989; Martínez et al, 1995, George et al., 2003). Loosanof (1968) and Shumway, (1996) reported similar behavior for oyster populations at different locations on the eastern coast of the United States, with variations in the amplitude and commencement of each stage, correlated to temperature and food availability. Seasonality in the reproductive period is a characteristic of temperate and cold climates, increasing in amplitude as latitude decreases, becoming more constant throughout the year as the equatorial latitudes are reached (Baqueiro, 1998).

The higher organic load that enhances reproduction at Malagana can evidently be correlated to urban and other sources of pollution, as is reflected in elevated fecal bacterial counts (Table 1) and heavy metal concentrations in the sediments (Table 2), posing a health risk. At Matacuaya, on the other hand, this problem was not detected, at least with regard to bacterial counts. However, the extent of heavy metal distribution in the lagoon and bio-accumulation by the oysters has still to be determined.

The differential reproductive strategy within the same water body has positive implications in recruitment. Given the availability of larvae throughout the year, they can take advantage of every occasion of adequate settlement conditions. Furthermore, they can be used to collect seed for replanting and aquaculture, with availability of mature organisms, or at least in an advanced stage of gametogenesis, throughout the entire year.

Table 3. F test for significant difference between gonadic stages for Crassostrea virginica from Malagana Point and Matacuaya Island, Pueblo Viejo lagoon, Veracruz, Mexico.

Stage.	N_1	$MEAN_1$	SD_1	N_2	$MEAN_2$	SD_2	**F**	P
I	48	2	3.284	42	1.75	1.511	**2.173**	0.005
II	31	1.292	2.136	48	2	2.126	**1.005**	>.10
III	35	1.458	2.395	11	0.458	0.779	**3.075**	0.005
IV	70	2.917	3.296	74	3.083	3.476	**0.948**	NO
V	36	1.5	4.149	51	2.125	2.802	**1.481**	>.05

1 Malagana, 2 Matacuaya

Table 4. F test for significant monthly difference of gonad development of Crassostrea virginica from Malagana and Matacuaya, Pueblo Viejo Lagoon, Veracruz, Mexico.

	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
$MEAN_1$	48	148	160	188	202	256	278	196	306	244	-	90
N_1	240	740	800	940	1010	1280	1390	980	1530	1220	-	450
SD_1	78.23	128.3	166.7	299.3	189.7	408.3	354.1	329.4	417.9	366.7	-	127.3
$MEAN_2$	162	176	202	164	204	242	230	276	306	240	-	234
N_2	810	880	1010	820	1020	1210	1150	1380	1530	1200	-	1170
SD_2	216.4	168.2	205.5	158.8	159	204.1	420.7	589.6	411.8	320.3	-	423.8
F	**2.766**	**1.31**	**1.232**	**1.884**	**1.193**	**2**	**1.188**	**1.79**	**1.015**	**0.873**	-	**3.33**
P	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	-	0.005

[1] Malagana, [2] Matacuaya

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References

Aldana Aranda D 1988. Situación actual de la pesquería de ostras en el Golfo de México y perspectivas de su maricultura. Memorias del IX Congreso Nacional de Zoología, Universidad Autónoma de Tabasco, México. Vol. **1**: 8-12.

Baqueiro CE 1998. Patrones en la dinámica poblacional y ciclo reproductor de moluscos bivalvos y gasterópodos de importancia comercial
en México. Doctoral Thesis. Mexico: CINVESTAV- IPN, Unidad Mérida. 389 pp.

Contreras EF 1985. Las lagunas costeras mexicanas. Centro de ecodesarrollo, Secretaría de Pesca, México. pp. 263.

Davis HC, Chanley PE 1955. Spawning and egg production of oysters and clams. Proc. Nat. Shellf. Ass. **46**: 40-51.

Galtsoff PS 1964. The American oyster Crassostrea virginica (Gmelin, 1791). Fish. Bull. **64**:1-480.

García E 1981. Modificaciones al sistema de clasificación climática de Köppen. Ed. Larios, México. pp. 252.

George Zamora A, Sevilla Hernández M, Aldana Aranda D 2003. Ciclo gonádico del ostión americano Crassostrea virginica (Lamellibranchia: Ostreidae) en Mecoacán, Tabasco, México. Rev. Biol. Trop., **51**, Supl **4**: 109-117.

Hernández AJ, Ortega S. 1988. Observaciones sobre la actividad reproductiva del ostión Crassostrea virginica (Gmelin, 1791) en el sistema lagunar de Mandinga, Veracruz, México. 1983-1984. Memorias del IX Congreso Nacional de Zoología, Univiersidad Autónoma de Tabasco, México. Tomo **1**: 111-117.

Lauckner G 1983. Diseases of Mollusca: Bivalvia. In: O. Kinne, editor. Diseases of Marine Animals Volume II. Introduction Bivalvia to Scaphopoda. Biologische, Anstalt Helgoland, Hamburg, Germany. pp. 477-520.

Loosanoff VL 1968. Maturation of gonads of oyster Crassostrea virginica at different geographical areas subjected to relatively low temperatures. Veliger **11(3)**:153-162.

Lucas A 1965. Recherche sur la sexualité des mollusques bivalves. Doctoral Thesis. France: Faculté des Sciences, Université de Rennes. 136 pp.

Luna LG 1968. Manual of histologic staining methods of the armed forces Institute of pathology. McGraw-Hill Book Company, New York. pp. 258.

Martínez I, Aldana Aranda D. Brulé T, Cabrera E 1995. Crecimiento y desarrollo gonadal del ostión Crassostrea virginica (Gmelin, 1791) (Mollusca Ostreidae), en la Península de Yucatán México. Avicennia. **3**: 61-75.

Navarrete Rosas OI 1989. Evolución gonádica y determinación del índice de fijación del ostión americano Crassostrea virginica (Gmelin, 1791) en el sistema lagunar Boca del Río-Mandinga, Veracruz. Mexico: Bachelors Thesis: ITMAR Veracruz. 90 pp.

Ortega HS, Arroyo JH 1988. Observaciones sobre la reproducción de Crassostrea virginica (Gmelin, 1791) en la laguna de Tamiahua, Veracruz, 1986-87. Memorias del IX Congreso Nacional de Zoología, Universidad Autónoma de Tabasco, México. Tomo **1**: 105-109.

Perkins FO 1993. Infectious diseases of mollusks, In: J.A. Couch, J.W. Fournie, edtitors, Pathobiology of Marine and Estuarine Organisms. USA: CRC Press, Boca Raton, Florida. pp. 255-287.

Ramírez GR, Sevilla M 1965. Las ostras de México. Secretaría de Industria y Comercio, Instituto Nacional de Pesca. Pub. 7. pp. 107.

Rogers NP, García-Cubas A 1981. Evaluación gonádica a nivel histológico del ostión Crassostrea virginica (Gmelin, 1791) del sistema fluvio lagunar. Atasta, Pom, laguna de Términos, Campeche, México. An. Inst. Cienc. del mar y Limnol. Univ. Nal. Auton. México, **8**:21-42.

SAGARPA 2004. Anuario estadístico de pesca 2001. Secretaría de Agricultura, Ganadería, Pesca y Alimentación. Mexico: Hoja electrónica de la Comisión Nacional de Acuacultura y Pesca: http://www.sagarpa.gob.mx/conapesca/index.htm

Sevilla M, Mondragón E 1965. Desarrollo gonádico de Crassostrea virginica (Gmelin, 1791) en la laguna de Tamiahua. An. Inst. Nal. Invest. Biológico Pesquero, México **1**:52-59.

Shumway SE 1996. Natural environmental factors. In: V.S., Kennedy, R.I.E. Newell, A.F. Eble, editors. The eastern oyster *Crassostrea virginica*. Mariland: Mariland Sea Grant Book. pp. 467-513.

Villanueva FS, Botello AV 1990. Metal Pollution in Coastal Areas of Mexico. Rev. Environ. Contam. Toxicol. **157**: 53-94.

Villanueva FS, Botello AV 1992. Metales pesados en la zona costera del Golfo de México y Caribe Mexicano: una revisión. Rev. Int. Cont. Ambient. **8(1**): 47-61.