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Evidence for *Crassostrea gigas* reproduction in the Bizert lagoon of Tunisia

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This study reports the first evidence of reproduction of *Crassostrea gigas* in the Bizert lagoon of Tunisia. The gametogenetic cycle of this species was examined from February 2002 to January 2003 for variation in maturation stage. Environmental parameters such as temperature, salinity, and chlorophyll a concentration were monitored. Initiation of gametogenesis took place in March when water temperature reached 14˚C. Ripe oysters were observed in May at a water temperature of 20˚C. Spawning began in June and reached a peak in September when water temperature ranged between 23˚C and 27˚C. During the latter period, oysters presented gonads with ruptured follicles and residual gametes. Spats, from 15 to 20 mm in length, were observed in November 2002 fixed under other oysters. Complementary studies have to be performed, but the present contribution could be of significance for the Tunisian oyster industry, which until now remains dependent on French spat importation.

Key words: bivalve, *Grassostrea gigas*, gametogenetic cycle, environmental parameters, Bizert lagoon.

INTRODUCTION

Oyster aquaculture industry is one of the oldest aquaculture activities worldwide. The evolution of this industry began in the 20th century after the establishment of reliable technologies (Lubet, 1991). In 2000, world production of aquaculture molluscs attained 10.73 millions of tones representing 30.15% of the aquaculture production in all sectors, which attained 35.58 millions of tones. The bivalve industry mainly concerned USA, China, Japan, Corea, France, Spain and The Netherlands (FAO, 2002). The major production was represented by oysters (37.37% of the total production in molluscs, 98.32% of which were *Crassostrea gigas*, 24.6% clams, 12.28% mussels, and 10.75% scallops Saint-Jacques (FAO, 2002).

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Crassostrea gigas in Tunisia has been farmed for the first time in the Bizert lagoon in 1972. Juvenile oysters were imported for the first time from Japan and then from France (Medhioub, 1993). This species has been studied by Gimazane & Medhioub (1979) and its growth was monitored in the Bizert lagoon for juveniles longer than 3 mm.

Gimazane & Medhioub (1979) and Medhioub & Zaouali (1988) have showed that different attempts for spat culture performed in 1977/1978 in the Bizert lagoon and in 1988 in the Ichkeul Lake, were not successful. Oyster production in Tunisia is since then dependent on the importation of juveniles from France. Indeed, the spat quantity imported from France in 2002 was about 665 Kg corresponding to a total cost of 5500 € and in 2003 was about 595 Kg which corresponded to a total cost of 5520 € (INS, 2003).

The purpose of this study was therefore to exam-

ine the seasonal pattern of the reproductive cycle of *C. gigas* in the Bizert lagoon. Understanding the effects of environmental conditions on oyster gametogenesis is considered essential for planning an oyster industry independent of juvenile importation.

MATERIALS AND METHODS

Study area and sampling

Bizert lagoon is located on the north of Tunisia, between 37° 08′ and 37° 14′ N and 09° 46′ and 09° 58′ E (Souissi, 1981). This coastal lagoon communicates directly with the Mediterranean Sea with an 8 Km long channel and with the Ichkeul Lake with a 5 Km long channel. The lagoon covers an area of 130,000 m^2 and the maximal depth is 12 m (Zaouali, 1979) (Fig. 1).

Samples of suspended oysters were collected from the oyster farm FMB (Ferme Marine de Bizerte), situated at 37° 09′ 38″ N and 09° 53′ 55″ E in the Bizert lagoon, at monthly intervals (from February 2002 to January 2003). Oysters were 12 months old, measured SE 6.69 ± 0.75 cm (maximum anterior posterior difference) and weighed 30.94 ± 10.82 SE g at the start of the trial.

Environmental parameter recording

The reproductive cycle can be influenced by a number of environmental conditions, such as temperature, salinity (Muranka & Lannan, 1984), and levels of nutrition which differ among localities (Chavez-Villalba *et al*., 2002). At the sampling site, temperature and salinity measurements were taken every month at the same point where the specimens were collected at 1 m depth. Chlorophyll a measurements were also performed every month at the site of culture and were evaluated using fluorimetric methods.

Histological studies

From February 2002 to January 2003 samples of 10 - 15 oysters were collected every month. The tissue of each oyster was removed from the shell, weighted and sectioned. Pieces of tissues were transversely cut from the gills and pulps, and also a few millimeters below them (Steele & Mulcahy, 1999). Pieces were then fixed in Bouin's solution and dehydrated through an ethanol series of increasing concentration. Dehydrated samples were cleared and embedded in paraffin following a standardized procedure (Lango-Raynoso *et al*., 2000). Sections (6 µm thick) were mounted on glass slides and stained with a solution of

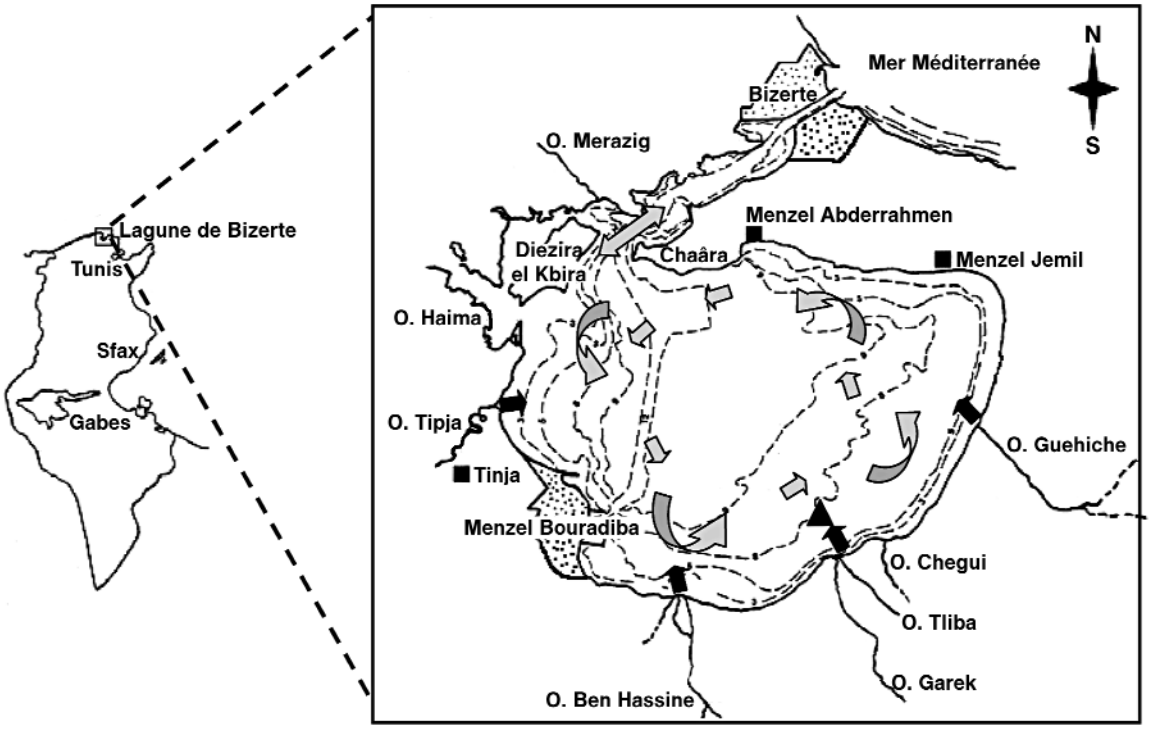

FIG. 1. Map of the oyster parc (FMB) in the Bizert lagoon (▲), with indication of water circulation according to Frisoni *et al*. (1986): ➡Influence of sea water, ➡ Influence of fresh water.

Groat's hematoxylin and eosin (Gabe, 1968). Each section of gonadal tissue was examined under the light microscope to assess the sex and the stage of gonadal development.

Biometry and condition indices

The valves of each individual were opened carefully and the flesh was removed from the shells. Before weighing, excess moisture was removed from all parts of the animal using absorbent paper. After recording the total wet weight, shell length, and visceral mass (gonad and digestive gland) wet weight, the tissues were oven-dried for 72 h at 60°C and the dry weight was determined using an electronic precision balance.

Fluctuation in the weight of the individuals may give information about the accumulation or depletion of the organic matter which depends on the stage of gonadal development. Benninger & Lucas's (1984) condition index of the oysters was calculated as the ratio of the dry weight of the soft parts/dry weight of the shell × 100 (Walne & Mann, 1975).

$$IC = \frac{\text{Dry weight of the soft parts}}{\text{Dry weight of the shell}} \times 100$$

RESULTS

Temperature, salinity, and chlorophyll a

According to the Tunisian Institute of Meteorology, during the period from 1990 to 1999 monthly means of temperature data of the air in Bizert varied between 11.18°C in January and 26.12°C in August. Water temperature, measured monthly, varied from a minimum of 10.9°C to a maximum of 28°C during the period of study. The highest monthly mean values occurred in August, while the lowest ones in January. The annual mean temperature was 19.7 ± 5.8°C (Fig. 2).

Salinity varied between 33 and 38‰ during the period of study. Salinity values were maximal during the summer (due to evaporation caused by the high temperature), but they decreased during the raining season (Fig. 2).

Chlorophyll a level displayed marked seasonal changes. Peaks were observed in May (2.4 μg l^{-1}) and November (1.2 μg l^{-1}) (Fig. 2).

Biometric measurements

Total wet weight, shell length, and visceral mass varied all over the year (Fig. 3). The visceral mass showed maximal values at the intense gametogenetic development, after which weight data decreased at the first partially spent in June and then values of visceral mass recovered in July and August. In September, visceral mass levels decreased again and then increased after the important phytoplankton production in autumn.

Condition index (CI)

The condition index data are shown in Fig. 4. The CI varied between 2.31 ± 0.73 and 4.96 ± 1.16. During the gametogenetic development, the CI remained high (4.31 ± 0.90) and it decreased (2.31 ± 0.73) at ripeness (June). The values of CI recovered between July and August, and then they decreased again in

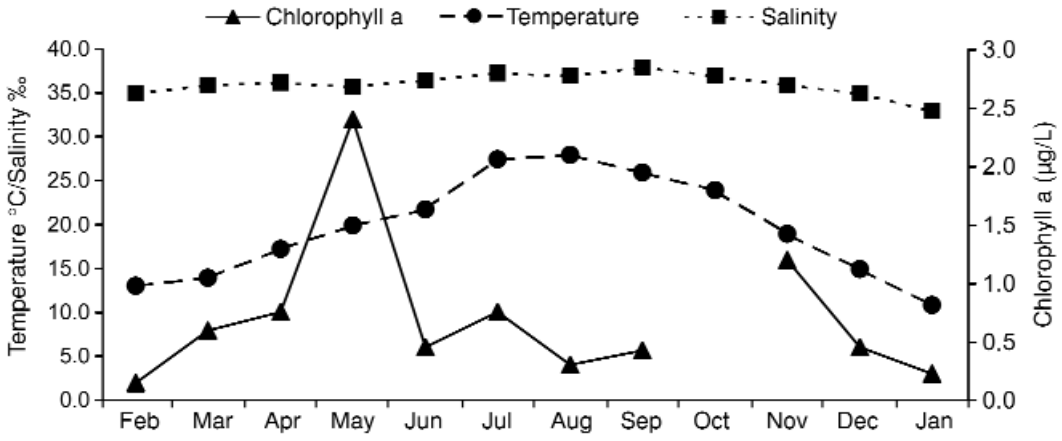

FIG. 2. Monthly variation of temperature, salinity, and chlorophyll a from February 2002 to January 2003.

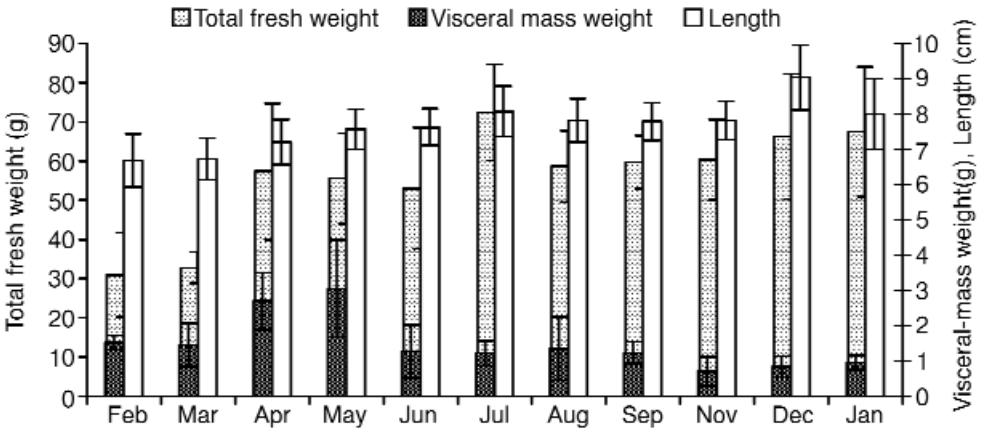

FIG. 3. Mean biometric data evolution of *Crassostrea gigas* in the Bizert lagoon (total wet weight, shell length and visceral mass wet weight). Bars represent standard deviation.

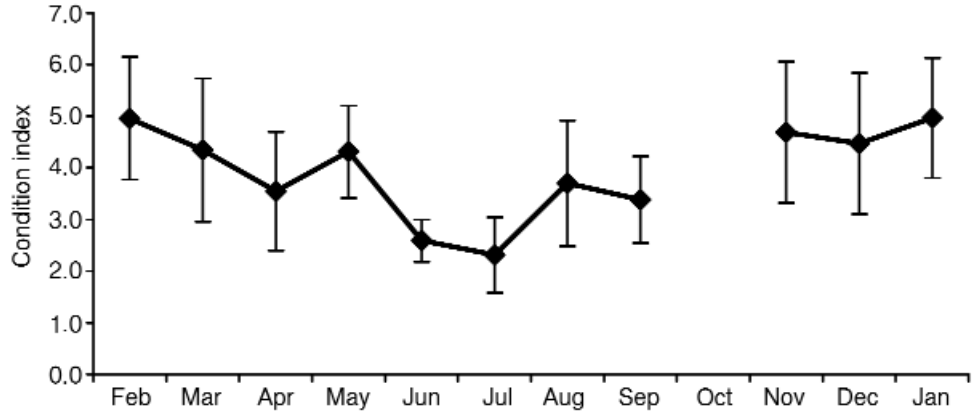

FIG. 4. Evolution of the condition index (CI) of *Crassostrea gigas* from February 2002 to January 2003.

September after massive spawning at the end of the summer. At sexual repose, CI levels increased again and reached maximal values essentially in winter (4.97 ± 1.16).

Sex ratio

Sex ratio varied throughout the year and the overall proportion of male and female oysters was approximately 1:1. The percentage of females decreased in May. Undifferentiated oysters were observed from November to February.

Gametogenesis

The reproductive cycle of *C. gigas* was studied in the Bizert lagoon from February 2002 to January 2003. Sex and gametogenetic stages were done according to the reproductive scale reported by Lubet (1959).

Stage 0: Undifferentiated gonads

This stage was observed in February, September, November and December of 2002 and in January of 2003 and it was characterized by a considerably developed connective tissue full of energy reserves with little follicular material. During this period, gonads were empty (sexual repose). There was no presence of follicles peripherally to the digestive gland making sex determination difficult (Fig. 5A).

Stage I: Gonad early development

This stage was established in March and April 2002. The interfollicular connective tissue decreased after gonia multiplication and expansion of the follicles, which contained oogonia or spermatogonia and primary oocytes or spermatocytes (Figs 5B and 6A).

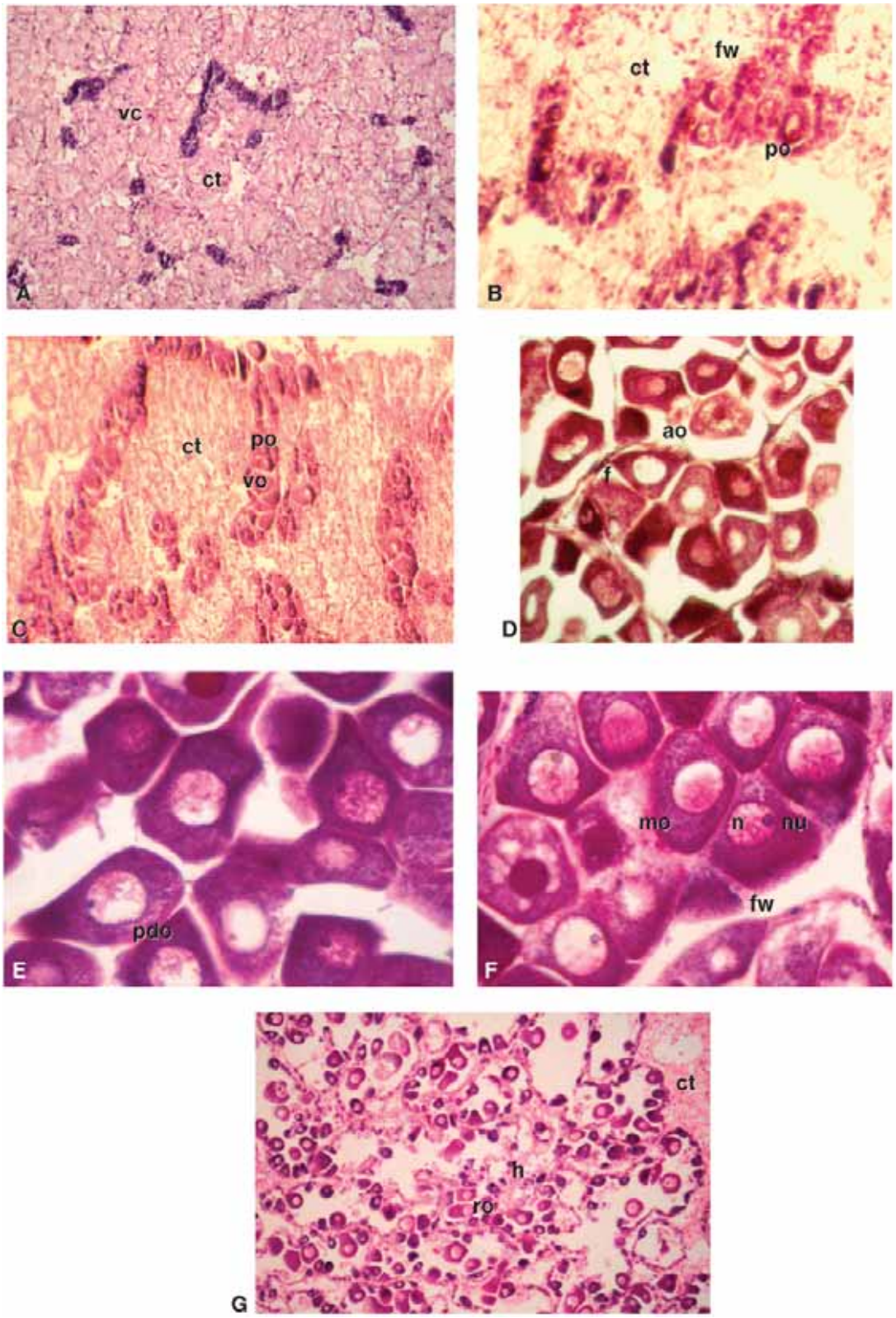

FIG. 5. *Crassostrea gigas*. Histological sections showing the female reproductive stages. (A) Sexual repose (100×); (B) Early activity (400×); (C) Late development; (D) Intense gametogenetic activity with adhering oocyte (100×); (E) Intense gametogenetic activity with peduncle oocyte (500×); (F) Mature oocyte (500×); (G) End of female sexual activity (100×) [adhering oocyte (ao), connective tissue (ct), vesiculated cells (vc), follicle (f), follicle wall (fw), mature oocyte (mo), primary oocyte (po), peduncle oocyte (pdo), residual oocyte (ro), nucleus (n), nucleolus (no)].

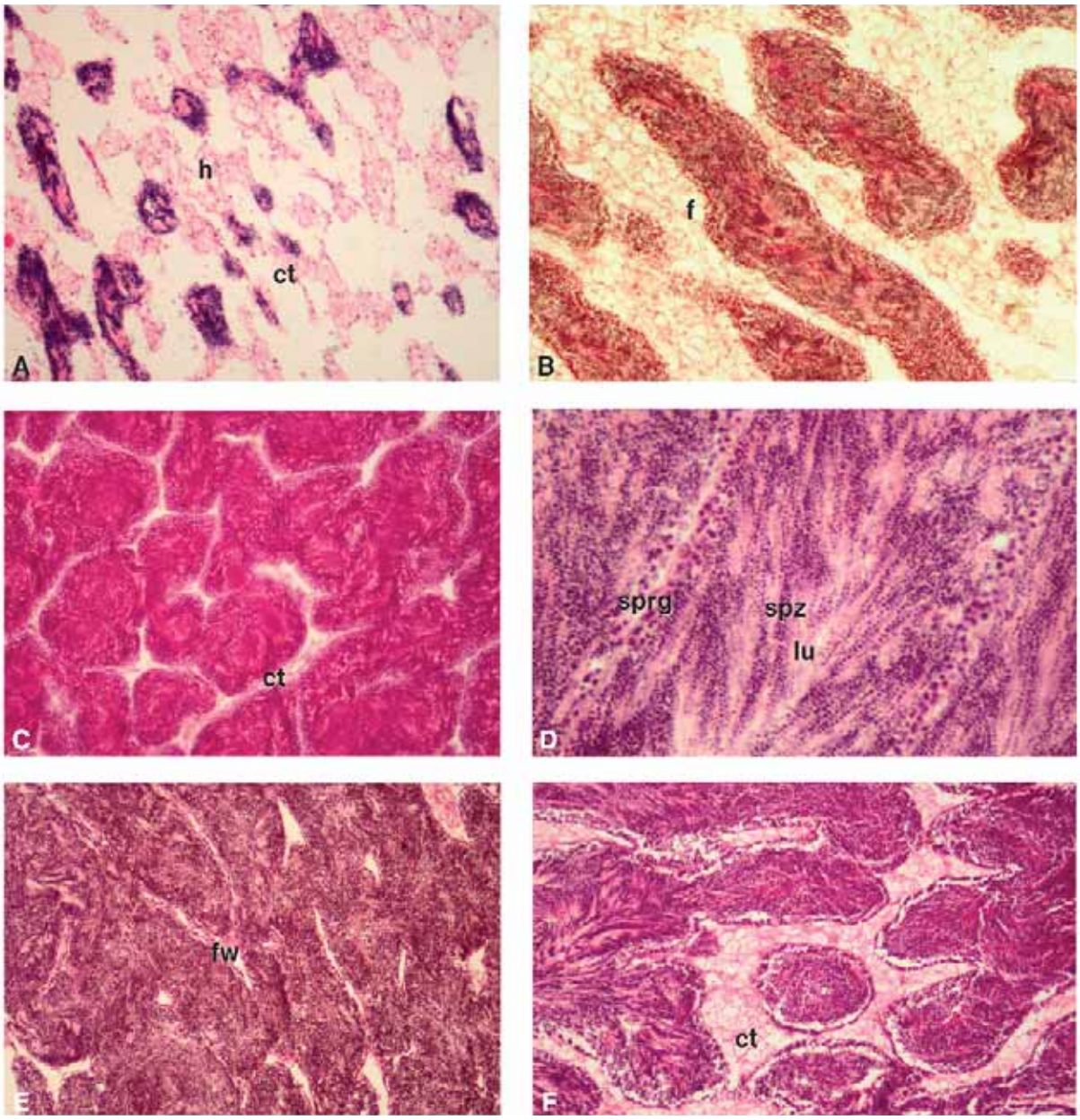

FIG. 6. *Crassostrea gigas*. Histological sections showing the male reproductive stages described in this study. (A) Early activity (100×); (B) Growing stage (100×); (C) Mature stage (100×); (D) Spermatozoa oriented with tails toward the follicle lumen (500×); (E) Homogenous appearance of follicles (100×); (F) End of male sexual activity (100×) [connective tissue (ct), hemocytes (h), follicle (f), follicle wall (fw), lumen (lu), spermatogonia (sprg), spermatozoa (spz), residual spermatozoa (rspz)].

Stage II: Gonad late development

This stage was detected in April 2002. An increase of the oocyte diameter was observed. Secondary oocytes and spermatocytes predominated in the follicles. Some spermatozoa could be observed (Figs 5C and 6B).

Stage III A: Intense gametogenetic activity

In May 2002, the interfollicular connective tissue disappeared. Male oyster gonads presented spermatids oriented toward the center of the follicle lumen, sometimes with a narrow band stage on the wall (Figs 6C and 6D).

The females exhibited a wide spectrum of oocyte sizes in all gametogenetic stages including some free oocytes.

1. Adhering oocyte: Oocytes were adhered to the follicle wall on a large surface. Their cytoplasm showed a darker coloration as it got

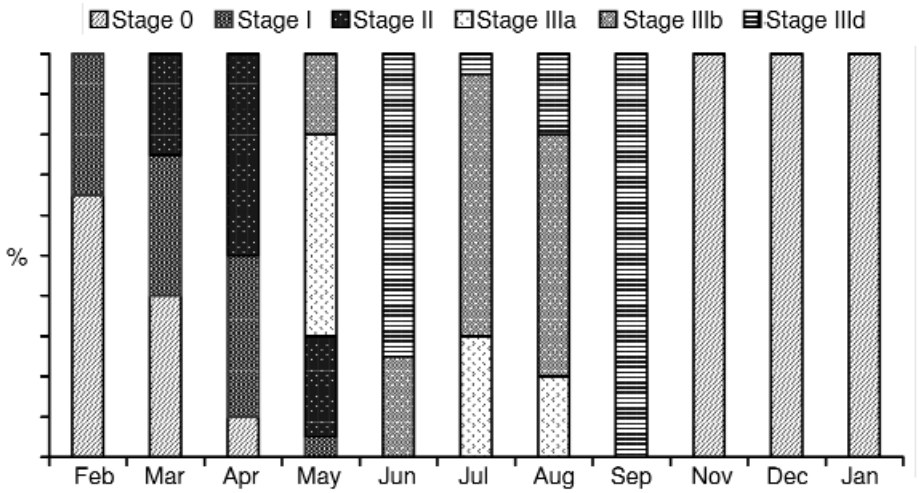

FIG. 7. Seasonal distribution of *Crassostrea gigas* at different stages of gonad development according to Lubet (1959).

richer in vitellus. Cells contained a distinct nucleus and nucleolus (Fig. 5D).

2. Peduncular oocyte: Adhesion of oocytes to the follicle wall was limited to a peduncle. Then cells became longer because of the vitell accumulation and the migration of the nucleus to the free extremity of the cells (Fig. 5E).

Stage III B: Gonad maturation

This stage was observed in May, July, and August 2002. The male oyster gonads presented a homogenous aspect after disappearance of the spermatids running toward the center of the follicle (Fig. 6E). The female gonads presented many mature oocytes with a polygonal shape that appeared to be free within the follicular lumen and able to expelle (Fig. 5F).

Stage III D: Spent gonads

This stage occurred in June and September 2002. Oysters presented ruptured follicles and residual gametes. Connective tissues reappeared between the follicles (Figs 5G and 6F).

The percentage distribution of different gametogenetic stages observed during the period of study is illustrated in Fig. 7. Gametogenesis began in March or April, after a period of a sexual inactivity during the months of November, December, and January. In February, more than 35% of the oysters were at stage I of gametogenetic development, and in March, 25% were at stage II. Gonads became ripe from May onwards. Although some individuals were at stage III B in May, the spawning activity (stage III D) was observed in June (75%), July (5%), August (20%), and September (100%). Between November, December and January, most of the oysters returned to the resting phase. We could therefore distinguish two phases in the gonadal cycle of *C. gigas* in the Bizert lagoon: a resting phase from November to January and gametogenesis during the rest of the year, including ripeness and spawning in the summer.

DISCUSSION

Several studies on the sexual maturation of bivalves have shown that the reproductive activity was influenced by environmental factors like temperature, food availability (Robinson, 1992; Mathieu, 1994) and pollutants (Paez-Osuna *et al*., 1995; Morcillo & Porte, 2000). Manipulation of physical (temperature) and nutritional (phytoplankton supply) factors may affect gonadal development, either by accelerating gametogenesis or by retarding gonadal maturation (Gallager & Mann, 1986).

Temperature has been often regarded as a factor controlling the pattern of the reproductive cycle in bivalves (Gribben *et al*., 2001) by accelerating the process of sexual maturation (Motavkine & Varaskine, 1989). The important role that this abiotic factor has on the reproductive cycle of *C. gigas* has been pointed out by Lubet (1976) and Shpigel *et al*. (1992). Lubet (1976) has showed that low temperatures induce lyses of the most aged oocytes, and that cold water induces retardation of metabolism due to decrease of food filtration. Gauthier-Clerc *et al*. (2001) have also established that at low temperatures gonadal activity is highly restricted. According to

TABLE 1. Temperature for gametogenesis initiation and gonadal maturity of *C. gigas* in different geographic locations

Location	Gametogenic initiation	Ripe oysters	Sources
Woods Hole (USA)	15-18˚C	18˚C	Mann (1979)
Taiwan, Korea, France, Portugal		19-25˚C	Bardach *et al*. (1972)
Sea of Japan (Hirochima, Matsushima)	16-22˚C		Ventilla (1984)
New Zealand	14-16˚C	18-25˚C	Dinamani (1987)
Marennes–Oléron Bay (France)	13-15˚C	17-18˚C	Fabioux *et al*. (2005)
This study (Tunisia, Bizert lagoon)	14-15˚C	20˚C	

Fabioux *et al*. (2005), multiplication of gonia occurred from November to March in Marennes-Oléron Bay (France) and gonial mitosis appeared to be clearly regulated by temperature (between 8 and 11˚C). In the present study, initiation of gametogenesis took place in March when temperatures in the Bizert lagoon were around 14˚C (Table 1).

We were able to observe gametogenesis of *C. gigas* in the Bizert lagoon since March 2002. Spawning began in June and September 2002, after which sexual repose took place. During this late gametogenetic stage, the interfollicular connective tissue occupied the majority of the gonadal tissue. This tissue represents the energy pool which accumulates metabolites during the winter and before the initiation of gametogenesis.

When gametogenetic activity started, gonia multiplication took place until April. During May, we observed mature gametes, spermatozoa oriented with their tails toward the follicle lumen, and ova with a polygonal form totally filling the follicle. The maturation of the oyster germ cells is highly affected by temperature, as suggested by Muranaka & Lannan (1984). This phenomenon was also observed until July and August indicating gonadic restoration (see Lubet, 1991). In the same study it was revealed in *C. gigas* from Arcachon (France) that gonad restoration in July/August preceded spawning in June/onset July and was followed by others at the end of August onset September. However, males presented in the same period (May) spermatozoa with their tails oriented toward the follicle lumen ready to expelle. In the Bizert lagoon, gametes were mature when water temperature reached 20˚C (May) (Table 1).

According to Lubet (1973), the connective tissue becomes reduced when the environment is hydrologically stable and rich in food. Many authors (Souissi, 1981; Anonymous, 1989; Mansouri, 1996) have reported that the Bizert lagoon is similar to a shallow wash-basin with a low rate of flow. This fact explains why our histological observations showed that *C. gigas* does not exhibit a real sexual repose period. Thus, during this time follicles are visible but very small and make sex determination difficult. Temperature is considered to be responsible for gametogenesis initiation, but it also plays a major role in spawning. Lubet (1976), showed that thermic shock had an impact on switching gamete release.

In the Bizert lagoon there is no tide, so spawning takes place thanks to other environmental factors like water warming in the summer (Lubet, 1959; Bejaoui, 1998 in *Mytilus galloprovincialis*; Ben Khedher-Dhaoui, 2001 in *Donax trunculus*). During the sexual cycle of *C. gigas* in the Bizert lagoon, release of gametes occurs also under the influence of the high temperatures of June and September (23 and 27˚C, respectively). During this period, oysters have gonads with ruptured follicles and residual gametes.

We have also noticed that sexual activity decreases after August. It has been generally assumed that resorption occurs after partial spawning events to "clean" the gonad and prepare it for a new cycle (Fabioux *et al*., 2005). Autumn has been often described as a critical period for bivalve conditioning (Wilson, 1981; Le Pennec *et al*., 1998; Robert & Gérard, 1999). Concerning this fact, Bejaoui (1998) has evidenced that the warm autumnal water results in stopping of the reproductive cycle of *Mytilus galloprovincialis* and conversion of the residual gametes to vesiculated cells accumulating metabolites during the sexual repose.

At the start of the spawning period (September) there was a rapid decrease of the tissue weights and of the condition index. At the beginning of autumn and in winter, the abundance of available food allowed a recovery of the tissue weight. This recovery was accompanied by an increase of the condition index and reached a maximum in winter which corresponds to the sexual repose during which reserves are accumulated in oyster tissues and are considered to

be used principally in gametogenesis and lost in spawning, but also to provide energy during growing conditions.

Temperature is not the only environmental factor which controls the reproductive cycle of *C. gigas*. Indeed, Muranka & Lannan (1984) and MacDonald & Thompson (1988) have displayed the impact of other ecological factors like salinity and chlorophyll a.

In this study we have remarked that there is no significant variation in salinity values during the whole period of the experiment. However, beginning of gametogenesis in *C. gigas* preceded chlorophyll a increase which began in November, and sexual maturity occurred simultaneously with phytoplankton impulse which began in spring. Kautsky (1982) has established that phytoplankton production induced reserve accumulation and gametogenesis switching. Robinson *et al*. (1992) and Samain *et al*. (1992) have shown that the quality of molluscan broodstock diet has an important effect on reproduction and quality of eggs. Also, the enriched microalgal diet during the reproductive conditioning of *Argopecten purpuratus* resulted in greater fecundity and better response to spawning (Martinez *et al*., 2000). Fabioux *et al*. (2005) demonstrated that optimal conditions of food level, temperature and/or photoperiod drive the reproductive internal clock of *C. gigas*, in particular for the regulation of gonial proliferation and germ cell maturation, both essential steps in oyster reproduction. Modification of these environmental parameters led to complete modification of the timing of *C. gigas* gametogenesis.

The results obtained in this study are in accordance with those previously obtained in other bivalve species like *Mytilus galloprovincialis* (Béjaoui, 1998), *Donax trunculus* (Ben Khedher-Dhaoui, 2001) and *Flexopecten glaber* (Ben Nakhla, 2002). Our results also showed that the Bizert lagoon can be adequate for bivalve reproduction mainly due to its hydrodynamic stability, warm waters and phytoplankton richness.

CONCLUSIONS

In this study we showed for the first time that there is a production of spat in the Bizert lagoon. According to our observations, the sexual cycle of *C. gigas* was completed successfully in the study site and new spats were observed, fixed under other oysters. Complementary studies have to be performed to show different parameters of spat fixation and larval development of this bivalve in the Bizert lagoon. This study can open new perspectives for the oyster industry in Tunisia. In fact, the Tunisian oyster industry could no longer be dependent on foreign spat importation, which represented until now an economic handicap to this industry.

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REFERENCES

Anonymous, 1989. Diagnostic préliminaire pour l'étude de l'équilibre écologique du lac de Bizerte. *Rapport final A.N.P.E.*: 72.

Bardach JE, Ryther JH, McLarney WO, 1972. *Aquaculture: The farming and husbandry of freshwater and marine organisms*. Wiley-Interscience, New York.

Béjaoui N, 1998. *Ecobiologie de la population de moules Mytilus galloprovincialis (Lamarck, 1819) du lac de Bizerte*. Thèse de Doctorat, Faculté des sciences de Tunis.

Ben Khedher-Dhaoui R, 2001. *Contribution à l'étude ecobiologique de Donax trunculus* (Linnaeus, 1758) *mollusque bivalve, dans le golfe de Tunis*. DEA, Faculté des sciences de Tunis.

Ben Nakhla L, 2002. *Contribution à l'étude biologique et à la valorisation du pétoncle Flexopecten glaber* (Linnaeus, 1758) *dans la lagune de Bizerte*. DEA, Institut National Agronomique de Tunis.

Benninger PG, Lucas A, 1984. Seasonal variations in condition, reproductive activity and gross biochemical composition of two species of adult clam reared in a common habitat: *Tapes decussatus* L. (Jeffreys) and *Tapes philippinarum* (Adam et Reeve). *Journal of experimental marine biology and ecology*, 79: 19-37.

Chavez-Villalba J, Pommier J, Andrimisez J, Povreau S, Barret J, Cochard JC, M Le Pennec, 2002. Broodstock conditionning of the oyster *Crassostrea gigas*: origin and temperature effect. *Aquaculture*, 214: 115-130.

Dinamani P, 1987. Gametogenic patterns in populations of pacific oyster, *Crassostrea gigas*, in Northland, New Zealand. *Aquaculture*, 64: 65-76.

Fabioux C, Huvet A, Le Souchu P, Le Pennec M, Pouvreau S, 2005. Temperature and photoperiod drive *Crassostrea gigas* reproductive internal clock. *Aquaculture*, 250: 458-470.

FAO, 2002. *Fishery statistics capture production*. Vol. 90/1. FAO Statistics series No 166.

Frisoni GF, Guelorqet O, Pethuisot JP, 1986. *Evaluation des potentialités aquacoles du lac de Bizerte*. Rapport FAO/MEDRAP, Tunis.

Gabe M, 1968. *Techniques histologiques*. Masson et Cie, Paris.

Gallager SM, Mann R, 1986. Growth and survival of larvae of *Mercenaria mercenaria* (L.) and *Crassostrea virginica* (Gmelin) relative to broodstock conditioning and lipid content of eggs. *Aquaculture*, 56: 105-121.

Gauthier-Clerc S, Pellerin J, Gagné F, Amiard JC, 2001. Vitellogenic activity in *Mya arenaria* (Mollusca bivalvia). *40th annual meeting of the Canadian Society of Zoologists, Sudbury*.

Gimazane JP, Medhioub N, 1979. Croissance de l'huître creuse *Crassostrea gigas* dans le lac de Bizerte à partir de naissains artificiels de petite taille. *Bulletin. Officiel. National des pêches de Tunisie*, 3: 95-97.

Gribben PE, Creese RG, Hooker SH, 2001. The reproductive cycle of the New Zeeland Venus clam *Ruditapes largillierti*. *Journal of shellfish research*, 20: 1101-1108.

INS, 2003. Institut national des statistiques de Tunisie.

Kautsky N, 1982. Quantitative studies on the gonad cycle, fecundity, reproductive output and recruitment in a Baltic *Mytilus edulis* population. *Marine biology*, 68: 143-160.

Lango-Reynoso F, Chavez-Villalba J, Cochard JC, Le Pennec M, 2000. Oocyte size, a mean to evaluate the gametogenic development of the pacific oyster, *Crassostrea gigas* (Thumberg, 1793). *Aquaculture*, 190: 183-199.

Le Pennec M, Robert R, Advendano M, 1998. The importance of gonadal development on larval productions in pectinids. *Journal of shellfish research*, 17: 97-101.

Lubet P, 1959. Recherches sur le cycle sexuel et l'emission des gamètes chez les mytilidés et les péctinidés (mollusques bivlves). *Revue des travaux de l'institut des pêches maritimes*, 23: 387-542.

Lubet P, 1973. *Exposé synoptique des données biologiques sur la moule Mytilus galloprovincialis* (Lmk, 1819). FAO, Rome.

Lubet P, 1976. Ecophysiologie de la reproduction chez les mollusques lamellibranches. *Haliotis*, 7: 49-55.

Lubet P, 1991. Bases biologiques de la culture des mollusques. In: Barnabé G, ed. *Bases biologiques et écologiques de l'aquaculture*. Lavoisier TEC & DOC, Paris: 99-210.

MacDonald BA, Thompson RJ, 1988. Intraspecific variation in growth and reproduction in latitudinally differentiated populations of giant scallop *Placopecten magellanicus* (Gmelin). *Biological bulletin*, 175: 36-371.

Mann R, 1979. Some biochemical and physiological aspects of growth and gametogenesis in *Crassostrea gigas* and *Ostrea edulis* grown at sustained elevated temperatures. *Journal of marine biological association of U.K.*, 59: 95-110.

Mansouri T, 1996. *Application de la télédétection et des systèmes d'information géographiques à l'étude du fonctionnement hydrologiques du lac de Bizerte et de son bassin versant*. DEA, Faculté des sciences de Tunis.

Martinez G, Aguilera C, Mettifogo L, 2000. Interactive effects of diet and temperature on reproductive conditionning of *Argopecten purpuratus* broodstock. *Aquaculture*, 183: 149-159.

Mathieu M, 1994. Endocrine control of carbohydrate metabolism in mollusc. In: Davey KG, Peter RE, Tobe SS, eds. *Perspectives in comparative endocrinology*. National Research Council of Canada, Ottawa: 471-474.

Medhioub MN, 1993. Aperçu sur la conchyliculture en Tunisie point de situation et réflexions. *Revue de l'institut national agronomique de Tunisie*, 8: 19-26.

Medhioub MN, Zaouali J, 1988. Captage de l'huître creuse *Crassostrea gigas* dans le lac de Ichkeul. *Bulletin de l'institut national des sciences et technologies de la mer*, 15: 47-60.

Morcillo Y, Porte C, 2000. Evidence of endocrine distribution in clam -*Ruditapes decussate*- transplanted to a tributyltin-polluted environment. *Environmental pollution*, 107: 47-52.

Motavkine PA, Varaskine AA, 1989. *Traduit du russe par Chantal Bellon-Humbert. La reproduction chez les mollusques bivalves: rôle du système nerveux et régulation*. IFREMER 10.

Muranka MS, Lannan JE, 1984. Broodstock management of *Crassostrea gigas*: environmental influences on broodstock conditioning. *Aquaculture*, 39: 217-228.

Paez-Osuna F, Frias-Espercueta MG, Osuna-Lopez JI, 1995. Trace metal concentration in relation to season and gonadal maturation in the oyster *Crassostrea iridescens*. *Marine environmental research*, 40: 19-31.

Robert R, Gérard A, 1999. Bivalve hatchery technology: The current situation for the pacific oyster *Crassostrea gigas* and the scallop *Pecten maximus* in France. *Aquaculture living resources*, 12: 121-130.

Robinson A, 1992. Dietary supplements for reproductive conditioning of *Crassostrea gigas* Kumamoto (Thumberg): I. Effects on gonadal development, quality of ova and larvae through metamorphosis. *Journal of shellfish research*, 11: 437-441.

Samain JF, Séguineau C, Cochard JC, Delaunay F, Nicolas JL, Marty Y, Galois R, Mathieu M, Moal J, 1992. What about growth variability for *Pecten maximus* production? *Océanis*, 18: 49-66.

Souissi N, 1981. Mécanismes de sédimentation et évaluation paléogéographique de la lagune de Bizerte (Tunisie) durant le quatérnaire récent. Thèse de Doctorat Spécialisée, Université Paul Sabatier de Toulouse, France.

Sphigel M, Barber BJ, Mann R, 1992. Effects of elevated temperature on growth, gametogenesis, physiology, and biochemical composition in diploid and triploid Pacific oysters, *Crassostrea gigas* Thunberg. *Journal of*

experimental marine biology and ecology, 161: 15-25.

Steele S, Mulcahy MF, 1999. Gametogenesis of the oyster *Crassostrea gigas* in southern Ireland. *Journal of marine biological association of U.K.*, 79: 673-686.

Ventilla RF, 1984. Recent development in the Japanese oyster culture industry. *Advances in marine biology*, 21: 1-57.

Walne PR, Mann R, 1975. Growth and biochemical composition in *Ostrea edulis* and *Crassostrea gigas*. In: Barnes H, ed. *Proceedings of the 9th European marine biology symposium*. Aberdeen University Press, Scotland: 587-607.

Wilson J, 1981. *Hatchery rearing of Ostrea edulis and Crassostrea gigas*, Ireland.

Zaouali J, 1979. Etude écologique du lac de Bizerte. *Bulletin de l'office national de pêche de Tunisie*, 3: 107-140.