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Mytilus galloprovincialis; GAMETOGENESIS OF A COLONY OF MUSSELS IN A FISH BREEDING ENVIRONMENT (ALBORAN SEA, M'DIQ, MOROCCO)

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Abstract- On the Mediterranean coast of Morocco (M'diq), there is a fish aquafarm. When mussel spat cross randomly this farming area, they attach themselves to the sides of the fish cages, making the latter undergo an incipient colonization. This article examines the reproductive cycle of the mussels aggregation concerning the *Mytilus galloprovincialis* species. The sampling was held over an unusually long period, from March 2009 to March 2011, we considered the following two biological parameters: the gonad index and condition index at a rate of one sample per month. We identified three periods of significant gametes emissions. In conclusion, given the constantly spawning activity, this area deserves further exploration in order to generalize the mussel farming in coexistence with the fish farming.

Keywords- *Mytilus galloprovincialis*, Reproduction, Condition index, Gonadic index, Histology

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Introduction

Morocco introduced a program for the development of the biological potential of its maritime space [1]. Called "Halieutis Strategy", this plan bases itself on a global and well-balanced management of the marine environment [1]. More exactly, the adopted strategy articulates around several themes, among which appears the fish farming, the basic component for a sustainable development. It should reach an annual production of 200 000 tons by 2020 [2,3].

With regard to this ambition, the culture of mussels could contribute partially substantial in the emergence of this pole; where from the necessity of knowing better their life cycle and the conditions of their existence. It is the subject of the present article dedicated to the understanding of the gametogenesis colony of the mussels *Mytilus galloprovincialis* in natural cohabitation with a breeding of fishes situated in the South of the sea of Alboran (M'diq, Morocco). In this sense, we proceeded monthly to the random collection of grown-up samples length superior or equal to 4 cm. at the level of two cages, then in their analysis to determine the sex, estimate the gonad activity and identify the mass spawning period.

It is important to underline here that the reproductive cycle on for a given individual interval between two successive spawning is subdivided into four phases: the initiation, the control of the gametogenesis, the maturation and the spawnings. Every species possesses an appropriate, genetic mode or not, to fit each of these four phases to the compulsory constraints in the middle of life [4-6]. The mussel *Mytilus galloprovincialis* is the only one of this kind to be present in Morocco [7]. It's distributing over littoral zone of Morocco from the Atlantic Ocean to Mediterranean Sea [7-9]. There are accumulation of studies on the reproduction of Mytilus for north Mediterranean waters including timing and duration of spawning [10-13]. However, the gamete production of *M. galloprovincials* depends on local environmental conditions [14].

Materials and Methods

Sampling Sites

The coast of M'diq appears as a continuation of lively cliffs and big bays developed in the mouth of rivers. It consists of two sectors of alluvial plains (north coast and south coast), split by the rocky outcrop of Cabo Negro (Kodiat Taifor). This one is a stressed and jagged promontory which rises until 332 m and drops almost vertically. The continental shelf is extremely narrow, not exceeding 3 km in width. Sediments which prevail on the whole continental shelf are arénites and muddy sands (bottom depth below 100 m), coastal sandy vases trend (100 m approx. to bottom depth), vases rich in mollusks (between 200 and 300 m), and sandy vases (from -300 m to -600 m). Isobaths are generally parallel to the coast with some curves. This isobaths mark a strong deep fall, for example in front of Cabo Negro, they decline as follows -5 m to 450 m of the bank, -10 m to 900 m and -20 m to 1650 m. The ground profile constitutes of a sandy cordon, a narrow plain followed by the first reliefs [Fig-1].

The studied zone possesses coordinates (35°41 ' N; 5°18 ' W). It is localized at the level of the farm of swelling of the sea bream and the sea perch. The domain occupies a parallelepiped of 76 000 m2; the fattening is made in 15 floating cylindrical cages in polyethylene,

14 having one diameter 12 meters for a fall of 10 m, and one of 8 m of diameter dedicated to the tests. This plan allows the passage of the stage alevin to the grown-up stage amounting to a commercial weight about 1 kg, at the end of eighteen months; what corresponds to ten thousand elements that is ten tons of fishes per cage. When wild spats cross at random this cattle-breeding area, they settle on the walls of the fish cages, and give birth to the colony of mussels (*M. galloprovincialis*) object of our study.

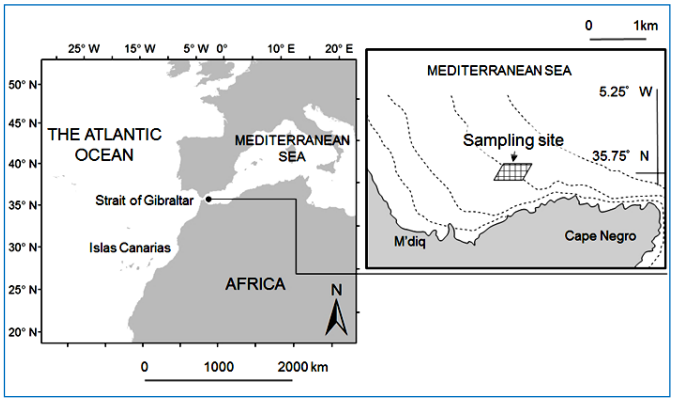

Fig. 1- Geographical study area of sampling site (M'diq), nearby Cape Negro (Koudiat Taifor); the sampling site was conductor in the depth range between 20 and 24 meters.

Sampling

The samples are taken and made along the outside surface of cages, by depths going from three to five meters. For that reason, the operation takes place when the sea is quiet. Furthermore, to improve the visibility, we choose the moment when the sun is high in the sky. The collections concern the period spreading out of March, 2009 until the same month of year 2011. The sampling contains forty to fifty individuals, each of length superior to 40 mm.

Mussels are placed in a cooled bowl, and immediately transported in the laboratory in isothermal conditions. Upon their reception, we proceed to the first treatments.

The temperature and the salinity are got by means of a conductimeter at 30 cm depth at stations.

Macroscopic Examination and Condition Index

We begin first of all to verify the vitality of the collected mussels. In particular, it is necessary to make sure that they are quite closed; each of them contains then three different parts: the shell, the intervalvular water (contained inside valves) and the flesh. We weigh then, in grams (g), every mussel to the closed state (± 0.01 g); this stage is followed by the measure of the caliber (length, width and thickness in ± 0.02 mm). Then, we proceed to the dissection. Upon the opening of the shell, we make sure of the membership of the mussel in *the Mytilus galloprovincialis* species thanks to the accolement which characterizes its posterior adductor muscles. We so establish a sample of thirty (30) elements. To calculate the condition index of every mussel, we use the following definition [15]:

$$Condition\ Index\% = \frac{[Weight\ of\ individual\ mussel\ \ flesh\,(g)]}{[Total\ weight\ of\ the\ shell\,(g)]}$$

Finally, we determine the condition index for every sample by making the average of the condition indexes of 30 individuals composing the sample.

The importance of the condition index of a given mussel, comes from the fact that it is an indicator of the crowning of its cycle of reproduction that is the spawning period.

Histology

A portion of gonadic tissue is cut at the level of the central part of the mantle of every individual. Cups are then placed in cassettes labeled according to the following nomenclature (ref.): Mg (*for M. galloprovincialis*), Nbr. of order (from 1 to 30), MQ (for M'diq), date.

Cassettes are then immersed in the Davidson solution during 24 hours, then dehydrated in baths of ethanol and finally filled with paraffin wax. Cups (2 µm of thickness in the microtome) are deposited on blades. After a time of drying of 12 hours, we color cups by the Hematoxyline-eosin Y. Finally, we cover blades by small strips maintained by an adhesive based on xylene.

The histological observation is made in the microscope, the swelling of X10 to X40. It reveals.

Gonadic Activity

The evolution of the gonadic activity during the sexual cycle is followed according to the protocol proposed by Lubet [16] and of whom will make use [17]. This method distinguishes seven stages: stage 0 (sexual rest); stage I (the beginning of the gametogenesis); Stade II (development of the gametogenesis and the appearance of gametes); stage IIIA (follicles gonadics filled with the mature gametes); stage IIIB (spawning), stage IIIC (restart of the gametogenesis); stage IIID (regression of gonadic follicles).

The gonadic index, proposed by Seed [18] for *Mytilus edulis*, is calculated from three parameters defined for every stage of maturity: index 1 (stage 0 and stage IIID); index 2 (stages I, II, IIIB and stage IIIC); index 3 (stage IIIA). These parameters (index 1, 2, 3) are called ratings.

To calculate the gonadic index, we proceed as follows: for each sample, the number of individuals corresponding to a stage of maturity is multiplied by the respective ratings. Products obtained are summed and the result is divided by the total number of individuals analyzed. We obtain a number (gonadic index), which ranges from 1 (when all individuals are at sexual rest) to 3 (when all individuals are matures).

Statistical Analyzes

Beforehand, let us say a word on the alternative of thirty (30) individuals as optimum of samples. The first reason results from the need for plausible extrapolation to the population of a result observed on a sample. Indeed, it turns out that on one hand the student test admits for upper bound 30 units [19]. R versions 2.15.2 (R. Core Team) under Windows are applied for all explanatory analyses and statistical tests in this study. So, the exploratory analysis, the analysis of the variance to compared with the seasons (One Way ANOVA) and the test of Kruskal-Wallis to highlight the seasonal differences relative to the gonadic parameter; the test of Chi 2 to estimate the gap from the sex-ratio observed in the distribution 1/1, with an equal significance level 0.05; also, we adopt a α risk of 5% for the calculation of the bilateral reliable interval of an average guessed at the level of the colony from the sampling. In a general way, all our hypotheses are tested at the α risk = 0.05.

Results

Salinity and Temperature

The salinity of coastal waters of M'diq varies between 35 and 37

during studied period. In 2009 (respectively on 2010, 2011) the lowest values appear in spring (Mars), (respectively at the end of the autumn in November and December), in winter (in January and February) [Fig-2]. In 2009 (respectively on 2010, 2011) the highest values appear in December, (respectively in September, March).

The temperature of the water undergoes according to the seasons of the sensitive variations. For three years 2009, 2010 and 2011, the lowest temperature 15°C is recorded in winter (February) and the highest 21°C at the end of the summer (September).

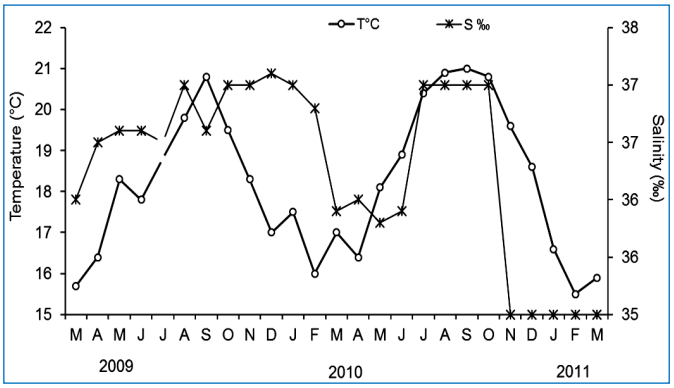

Fig. 2- Seasonal variation of the temperature and salinity of the sea water in the site M'diq, the measures are made on surface in 30 cm of the depth between March 2009 and March 2011.

Sex-ratio

The number of females (51.73%) is upper among males (46.53%); the percentage of sterile mussels or in indefinite sex represents 1.46% of the global sampling. It should be noted two cases of hermaphroditism, met respectively in July and August of year 2010. The calculated sex-ratio is not significantly different from the theoretical sex-ratio 1/1 (*p*-value = 0.131); the cases of hermaphroditism are counted as females [Table-1].

Table-1- Chi-square test on the sex ratio of adult mussels Mytilus galloprovincialis in the study M'diq site

Independent variable	Sex	Observed number	theoretical number	ddl	χ^2	p-value
M'diq	Female	390	369.5	1	2.274	0.131
	Male	349	369.5			

The sex-ratio differs from one month to the next: females present an ascendancy to September and December of year 2009, as well as in February and July, 2010. However, males are majority at the beginning of the winter, 2010. Seven individuals of sex undecidable are observed in August, 2010.

Cycle of Reproduction

The phase of initiation (stage I and II) appears [Fig-3](A), several times a year, according to a rather important proportion (≥ 20% of the sampled mussels). So, for 2009 in October (43%) and in December (30%); for 2010 in July (20%), in November (26%) and in December (30%); for 2011 in January (50%) [Fig-4].

The phase of maturity (stage IIIA) [Fig-3](B) is present all year round; but it is particularly marked some months (≥ 30% of the sampled mussels). So, for 2009 in May (53%) and in December (33%); for 2010 in February (46%), in May (53%) and in November (46%); for 2011 in February (56%).

There is a constant emission of gametes (stage IIIB) [Fig-3](C), with peaks superior to 50%. So, for 2009 in March (70%), in July (60%) and in November (53%); for 2010 in January (60%), in March (66%), in June (70%), in September (80%) and in October (60%). This durability is realized because of the relay assured by the stage IIIC (restart) which immediately takes place after the spawning [Fig-3](D).

Because of the relay which we have just evoked, the ultimate phase of the cycle (stage IIID and 0) is rare to observe; nevertheless, it's sometimes affects notable proportions (≥25%) of sampled mussels. So, for 2009 in September (26%) [Fig-3](E), and for 2010 in August (33%) when the resting stage present 13% [Fig-3](F).

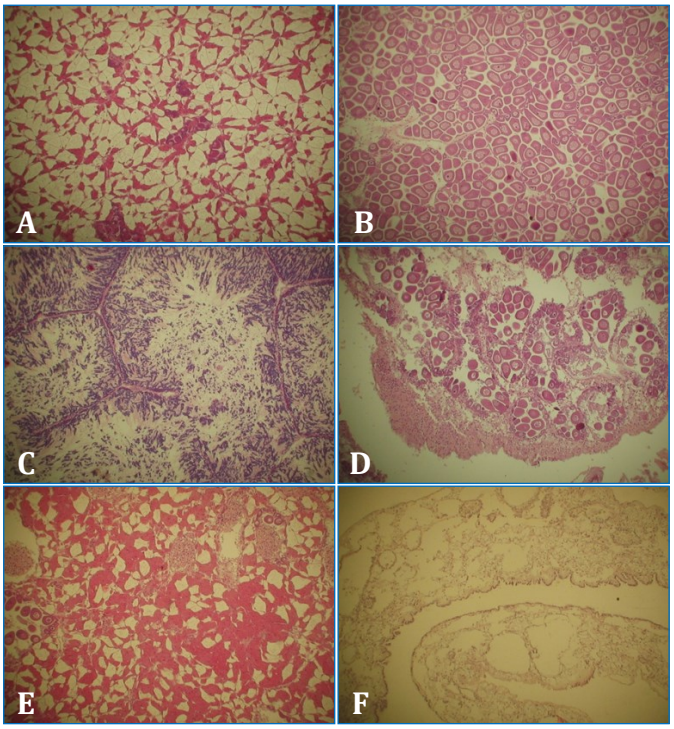

Fig. 3- Photomicrographs (X10) of gonads at different stages in reproductive cycle. **(A)** Resting, stage 0, ref. Mg 16 MQ 26.8.09.; **(B)** Appearance of the gametogenesis, stage I female, ref. Mg 14 MQ 17.12.09; **(C)** Fully ripe, stage IIIA female, ref. Mg 27 MQ 2.3.11; **(D)** massive spawning, stage IIIB male, ref. Mg 1 MQ 21.1.10; **(E)** Restart of the gametogenesis, stage IIIC female, ref. Mg 25 MQ.24.3.09.; **(F)** Endangered follicles and renewal of the connective tissue, stage IIID female, ref. Mg 22 MQ 17.12.09.

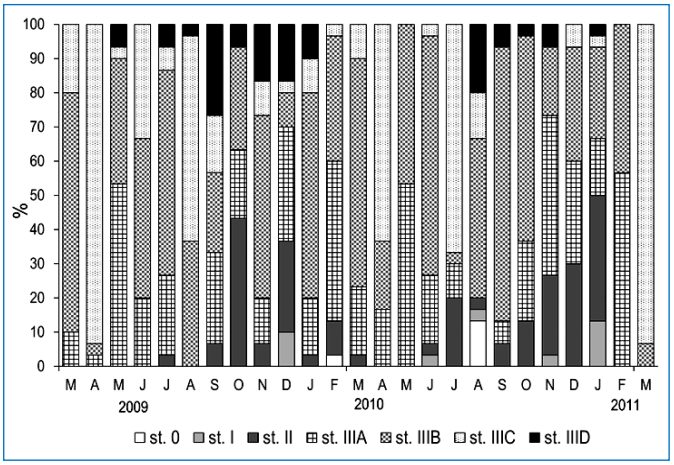

Fig. 4- *Mytilus galloprovincialis*; Monthly distribution of the stages of reproductive cycle studied between March 2009 and March 2011.

Gonadic Index

The gonadic index possesses a band of variations between 1.6 and 2.53. Maxima [in May 2009; in February, May and November on 2010; in February 2011] indicate an advanced level of maturity (stage IIIA). On the contrary, the minima of the index (on August, 2009 and 2010) correspond to the sensitive presence of the stage IIID and 0. Over the period of study, the average is equal to 2.15, without notable dispersal, standard deviation of 0.18 [Fig-5]. The boxplots by season let's suppose that the gametogenesis is dynamic practically all year long [Fig-6]. The Kruskal-Wallis test confirms this durability: there is, with regard to the seasons, no significant differentiation of gonadic activity (*p*-value = 0.48).

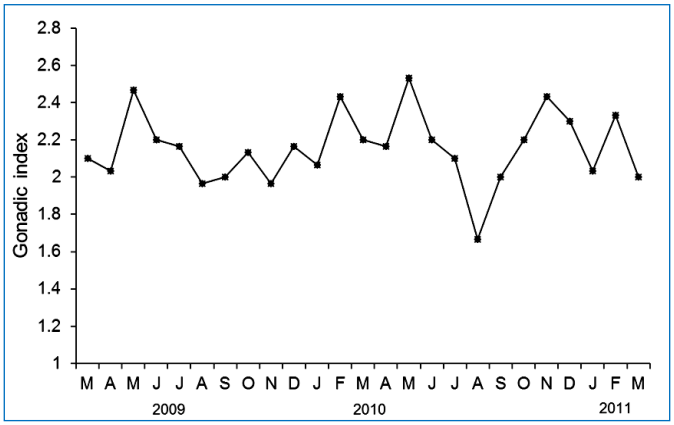

Fig. 5- Monthly Variations of the gonadal index of *Mytilus galloprovincialis* at M'diq.

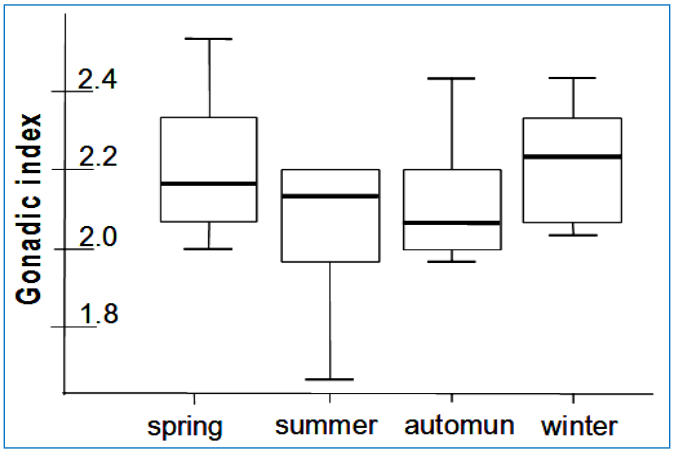

Fig. 6- Boxplot Highlighting the seasonal distribution of gonadic index.

Condition Index

The condition index oscillates in a regular and progressive way around an average of 45.57%. The maximum (59%) is reached in February 2011 and minimum (32%) in August 2010 and January 2011 [Fig-7]. We notice a uniformity (47%) from December 2009 till April 2010. The seasonal standard deviations are relatively moved closer and boxplot suggests that there is normality of the distribution with regard to every season. Finally, the analysis of the variance (One Way ANOVA) confirms the no seasonal differentiation of the condition index (*p*-value = 0.641) [Fig-8].

Overall timeseries of GI and CI had significant positive correlation (r = 0.43, *p*-value < 0.05). The decline of the average CI coincides with decline of GI, associating to spawning activities. Especially, CI and GI from spring and the summer 2010 associated each other. The linear correlation from May 2010 to August 2010 were 0.63 (*p*-value < 0.05) corresponding to the potential spawning. Gametic maturity (stage IIIA) was observed the months when they are observed the prominent values of CI. In February 2011, the maximum CI associated to dominance of the individual *M. galloprovincialis* at the stage IIIA.

Finally, before ending, we show, for information purposes, an example of inference on the colony from a sampling, as already evoked at the beginning of the paragraph 2.5. For that purpose, we consider the collection of February 2011, the condition index is 59%; it is the average of the indexes condition of 30 individuals composing the sampling; the corresponding standard deviation is equal to 15.3; then, we can assert that the average of the condition index of the population of mussels, having a length superior or equal to 40 mm, has 95 chances on 100 to be on the interval (53, 65).

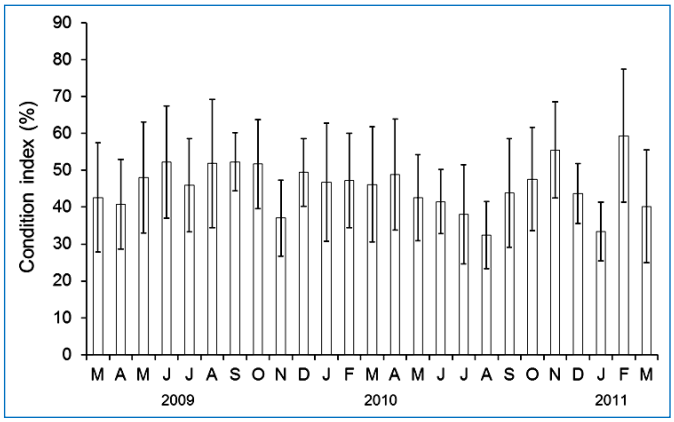

Fig. 7- Evolution of condition index and the corresponding monthly standard deviation of *Mytilus galloprovincialis* in the site.

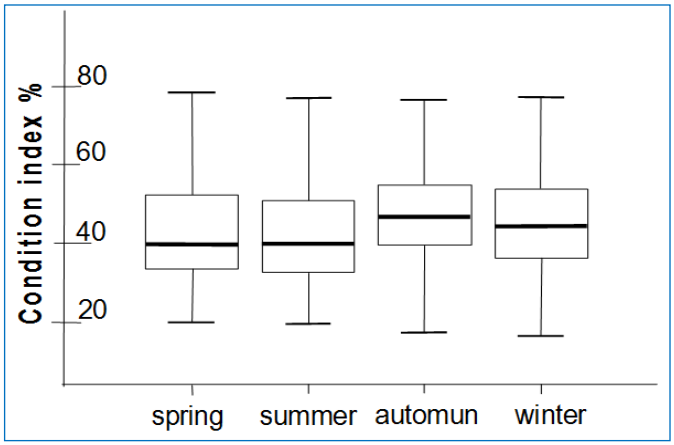

Fig. 8- Boxplots by season of condition index.

Discussion

The cages of the fish site of M'diq were the object of a natural colonization by wild spats of the species *Mytilus galloprovincialis*. The study of the corresponding gametogenesis shows that this colony is characterized by Three peak of spawning occurred entire the cycle. They are result of continuous reproductive cycle through entire seasons and it does not have specific inactive phase of gonadal development as a population.

The gonadic index and the condition index evolve in phase at the rate of the seasons; they are at the most or at least in the same periods of the year. The gametogenesis progressed in autumn and

early winter from September to January. Ripening gonad is in February and the mass of spawning stayed in spring and extended until August, because of gonad restoration. It should be noted that, during the period of gametogenic development in autumn (November) and spring (May), the mussels showed two peak of spawning after an important gonad ripeness. It was called imperfect ripeness by Villalba [11]; which the mantle of mussels were characterized by the presence of follicles occupied not all spaces of mantle but present only ripe gametes. During intensive spawning between May and August, the process of both emission and rapid redevelopment of gametes may take place simultaneously. August and September are characterized by transition between two periodic cycles.

The timings of the spawning corresponds to the spawning timings of Mytilus in north-west of Spain [11] and also in temperate zones [18,20-22]. We speculate that the conditions of surrounding water such as food availability based on high primary productivity [23] and preferable thermal conditions in this site allows the template *M. galloprovincialis* continuously reproduce. It should be noted that the resting stage of some mussels coincides with a significant increase in the temperature (21°C); correlation already raised by Fearman & Moltschaniwskyj [24]. In any case, the salinity, relatively stable, does not seem to play a particular role in this slowdown as reported by Lemaire, et al [23]. The contentious reproduction of Mytilus has been observed in tropical or subtropical zones [25].

Finally, the exceptional fertility of the studied colony must be connected with the proximity of fishes in intensive breeding. We think that the fattening also benefits mussels: their spawning mechanism works in tense flow [6].

Conclusion

In conclusion, given the durability of the spawning activity, this region would deserve an exploration more deeper to generalize the mussel breeding in cohabitation with the fish farming there.

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Conflict of Interest : None declared.

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